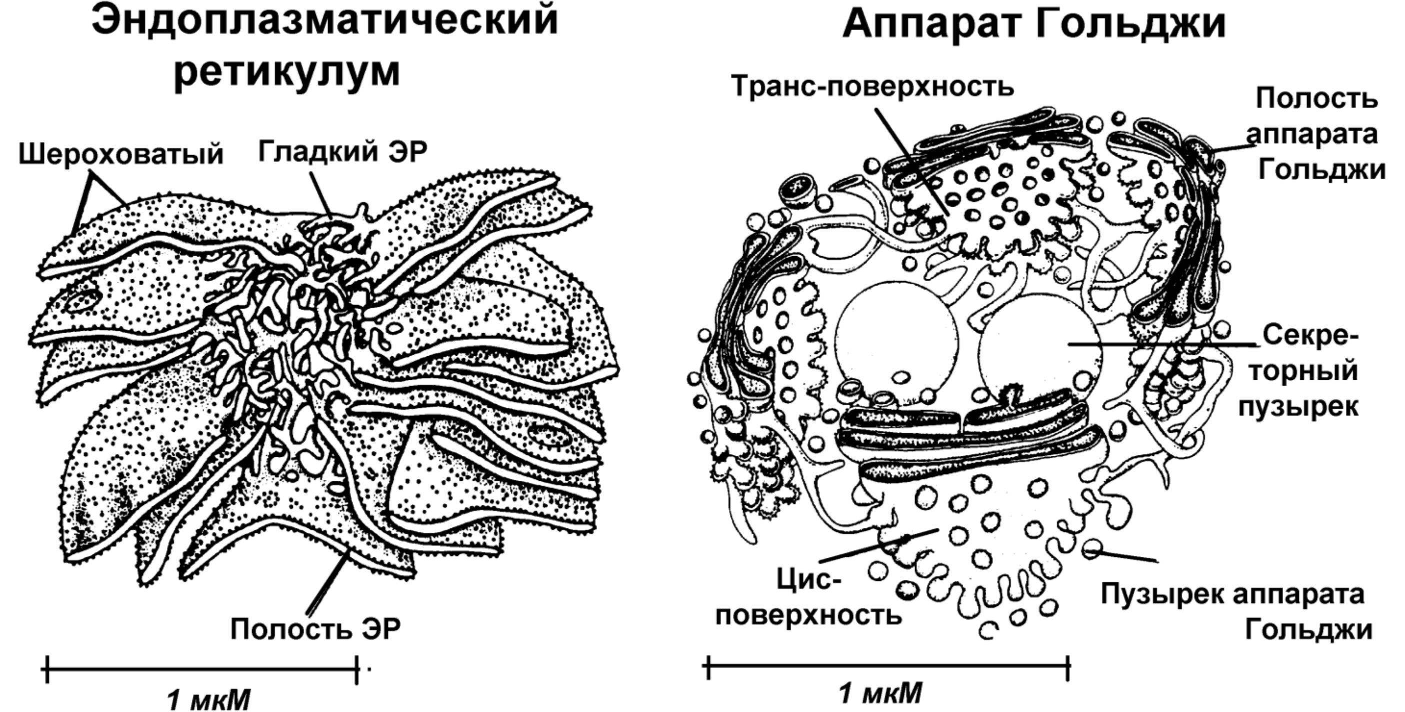
# Синтез гликопротеинов и протеогликанов у эукариот с участием ЭР и ап.Гольджи



# Folding in ER

- Oxidative folding, S-S- bonds → PDI (protein disulfide isomerase)
- Chaperons: Hsp70, hsp90, BiP, Grp94
- cis-trans isomerization of proline imidic peptide bonds: peptidylpropyl isomerase (PPI)
- Calnexin (binding for unfolded)
- Calreticulin (Ca-binding
- Role of calcium

# Protein Glycosylation in ER

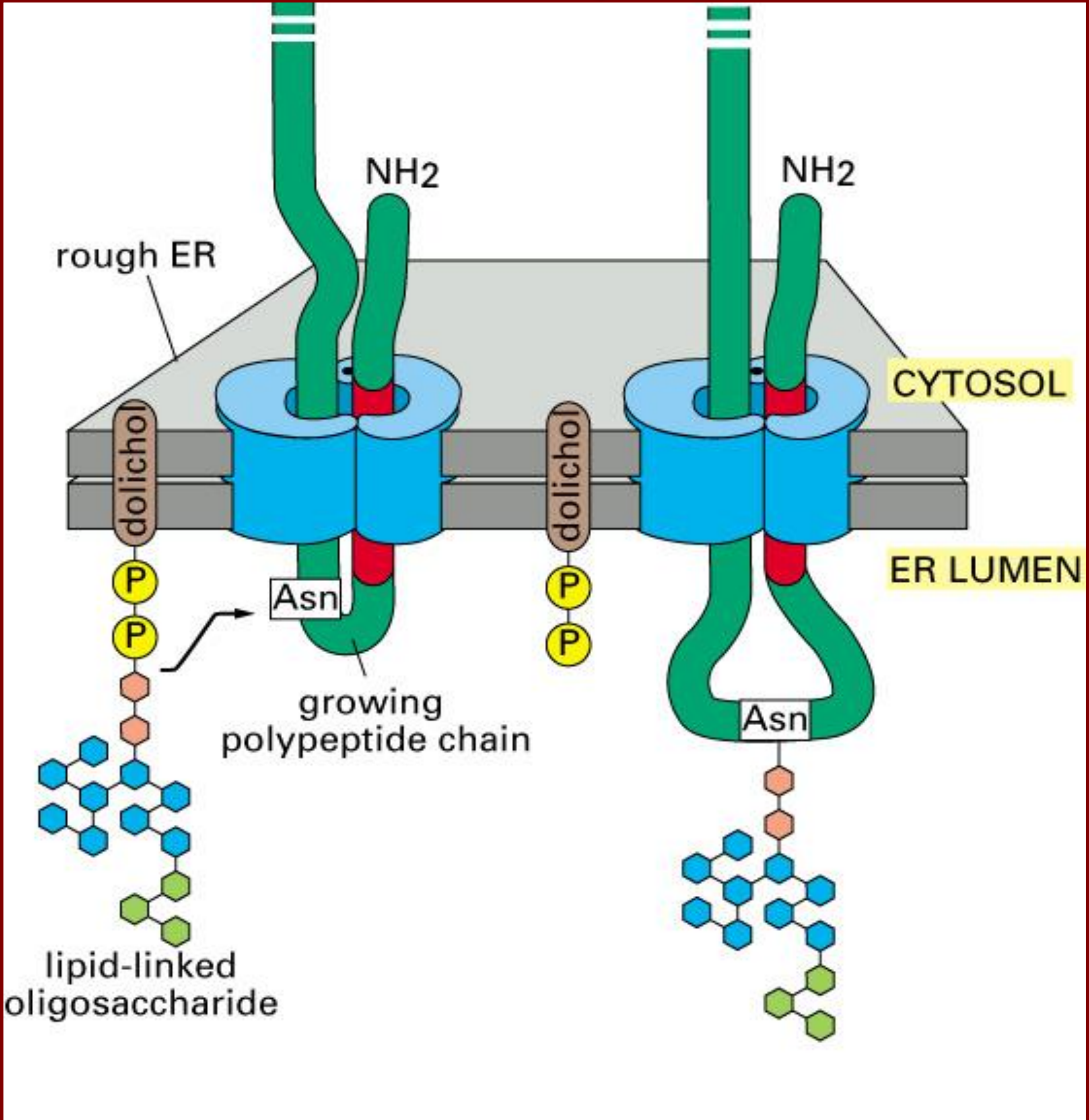
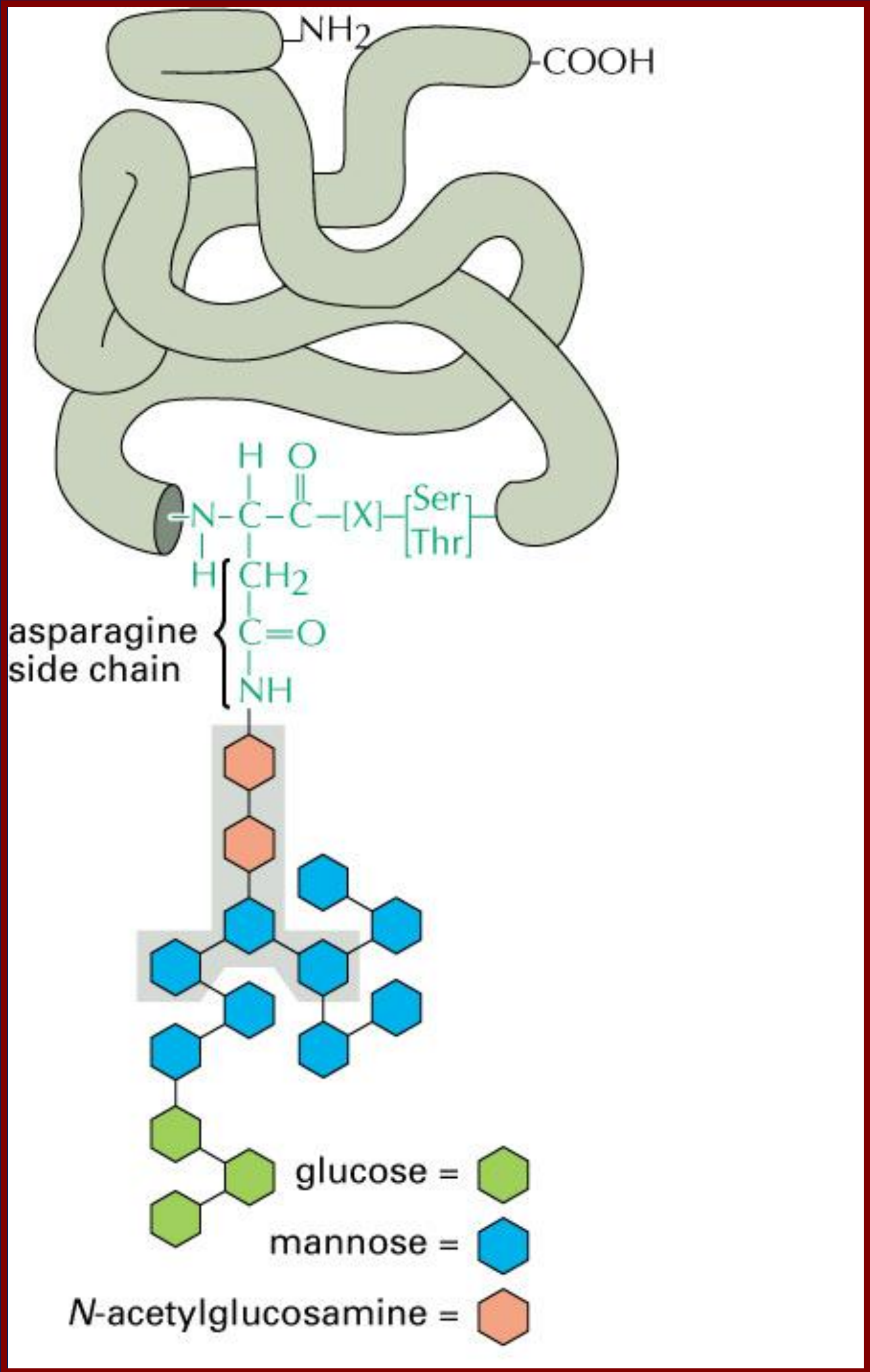


Figure 12–52. Molecular Biology of the Cell, 4th Edition.

# Finally modification of oligosaccharide



# Oligosaccharide synthesis in ER

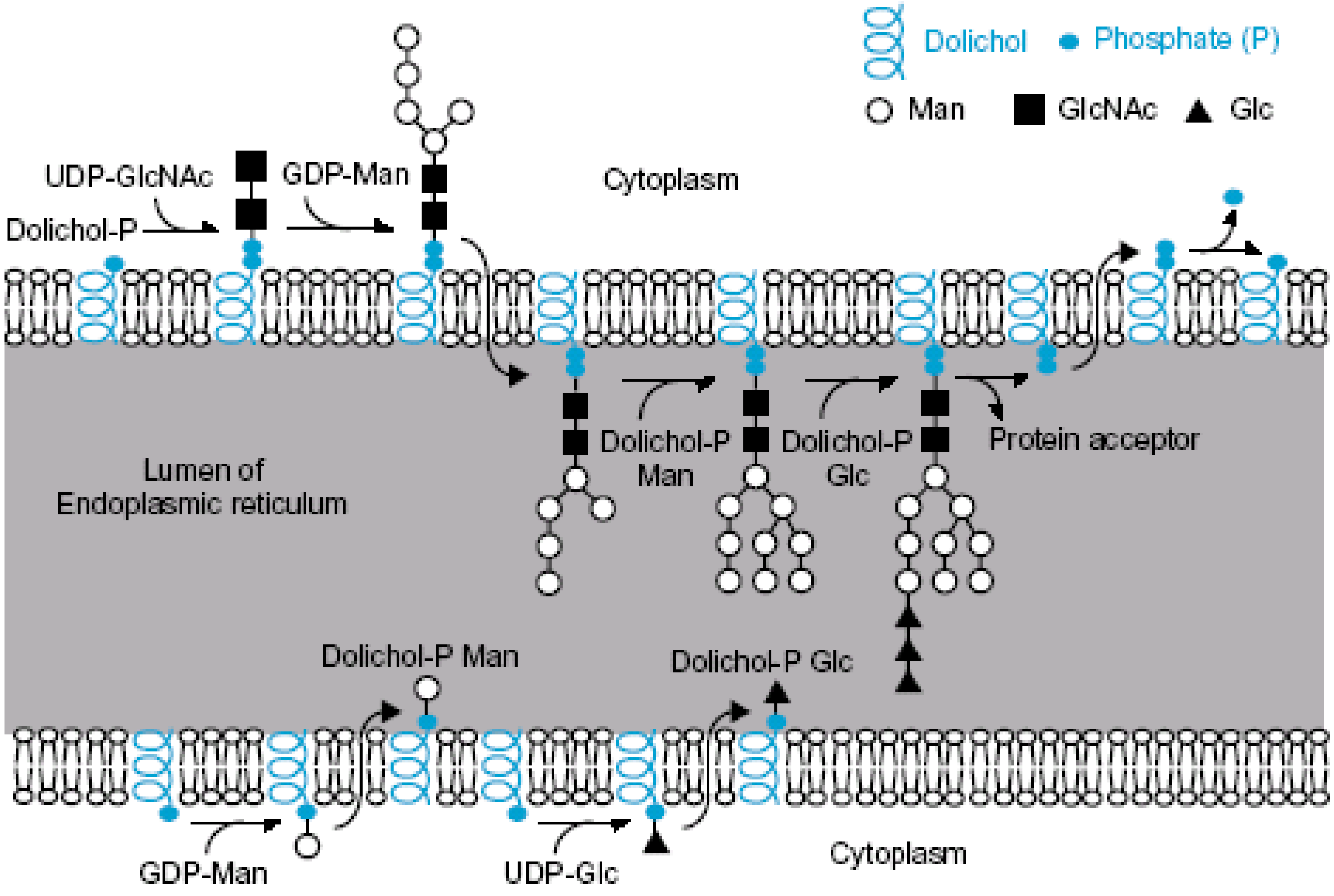
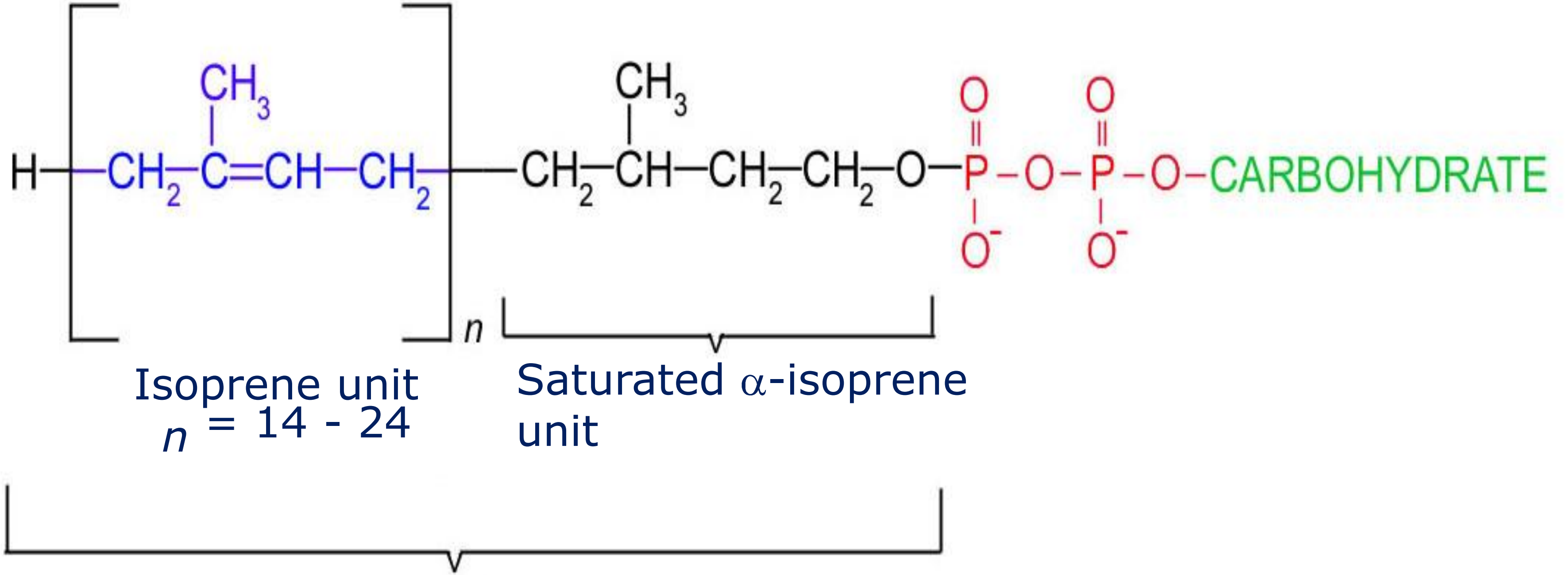


**Figure 2.4** Pathway for generation of the dolichol-linked oligosaccharide donor for protein N-glycosylation.

# Dolichol as anchor



$$H-\left[CH_2-\overset{CH_3}{\overset{|}{C}}=CH-CH_2\right]_n-CH_2-\overset{CH_3}{\overset{|}{CH}}-CH_2-CH_2-O-\underset{O^-}{\overset{O}{P}}-O-\underset{O^-}{\overset{O}{P}}-O-CARBOHYDRATE$$

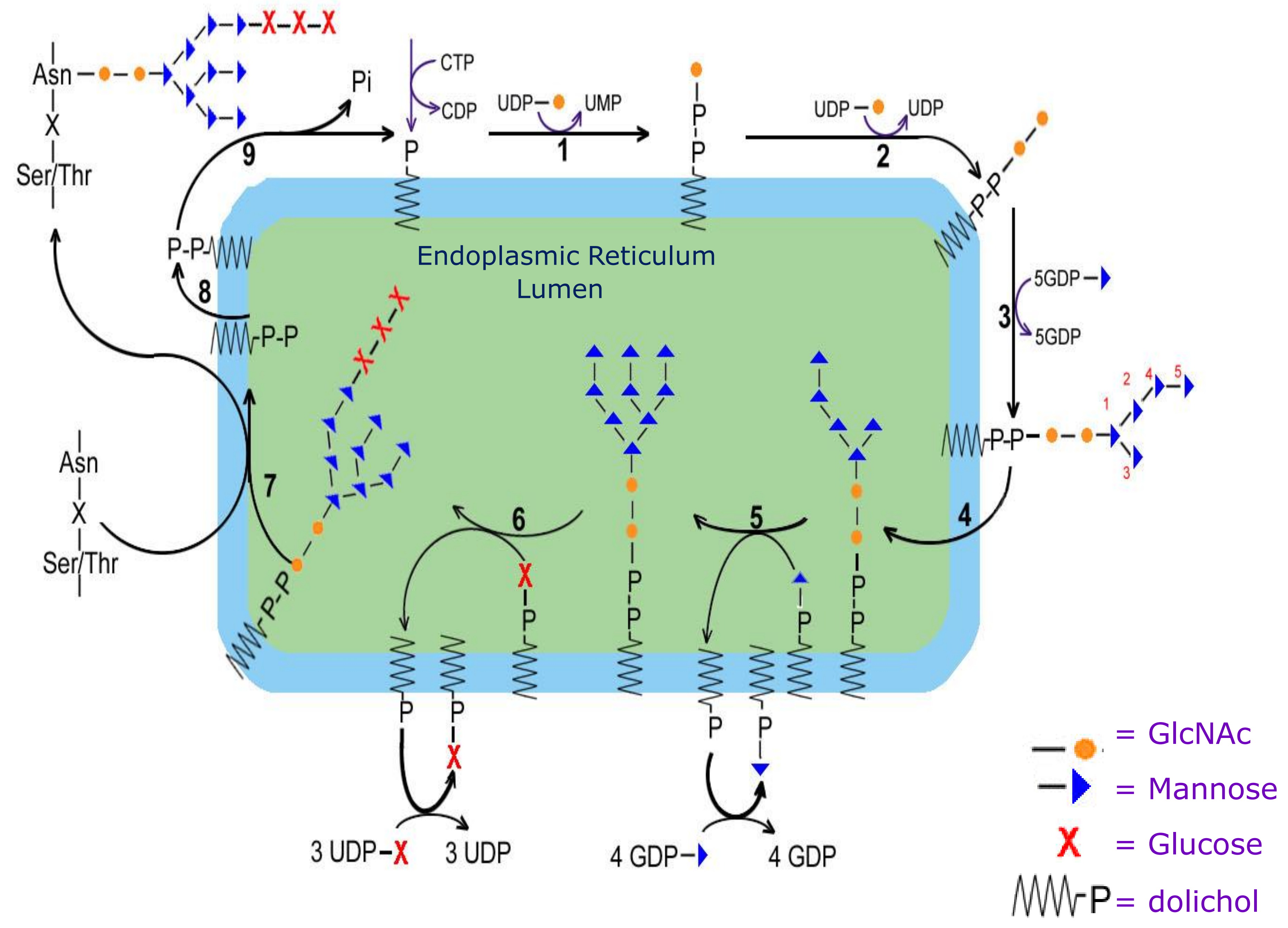
Isoprene unit
$n = 14 - 24$

Saturated α-isoprene unit

Dolichol

The carbohydrate precursers of N-linked glycosides are sythesised as dolichol phosphate glycosides.

# Pathway of Dolichol-PP-Oligosaccharide Synthesis



# Golgi Structure:
## A system of cisternae and associated vesicles

- **Transitional elements** or **ERGIC**
- **cis-, medial, trans cisternae**
- **Trans-golgi network**
  - Sorting to: Lysosomes, secretory vesicles, cell membrane
  - **Vesicles**: anterograde and retrograde transport
  - sorts protein to next destination. also a series of tubules
- **Peripheral scaffold** of proteins
  - Support structure consisting of:
    - Spectrin, ankyrin, actin
    - Golgi Matrix – (mitosis)

**The Golgi Apparatus has two major functions:**

1. Modifies the N-linked oligosaccharides and adds O-linked oligosaccharides.

2. Sorts proteins so that when they exit the trans Golgi network, they are delivered to the correct destination.

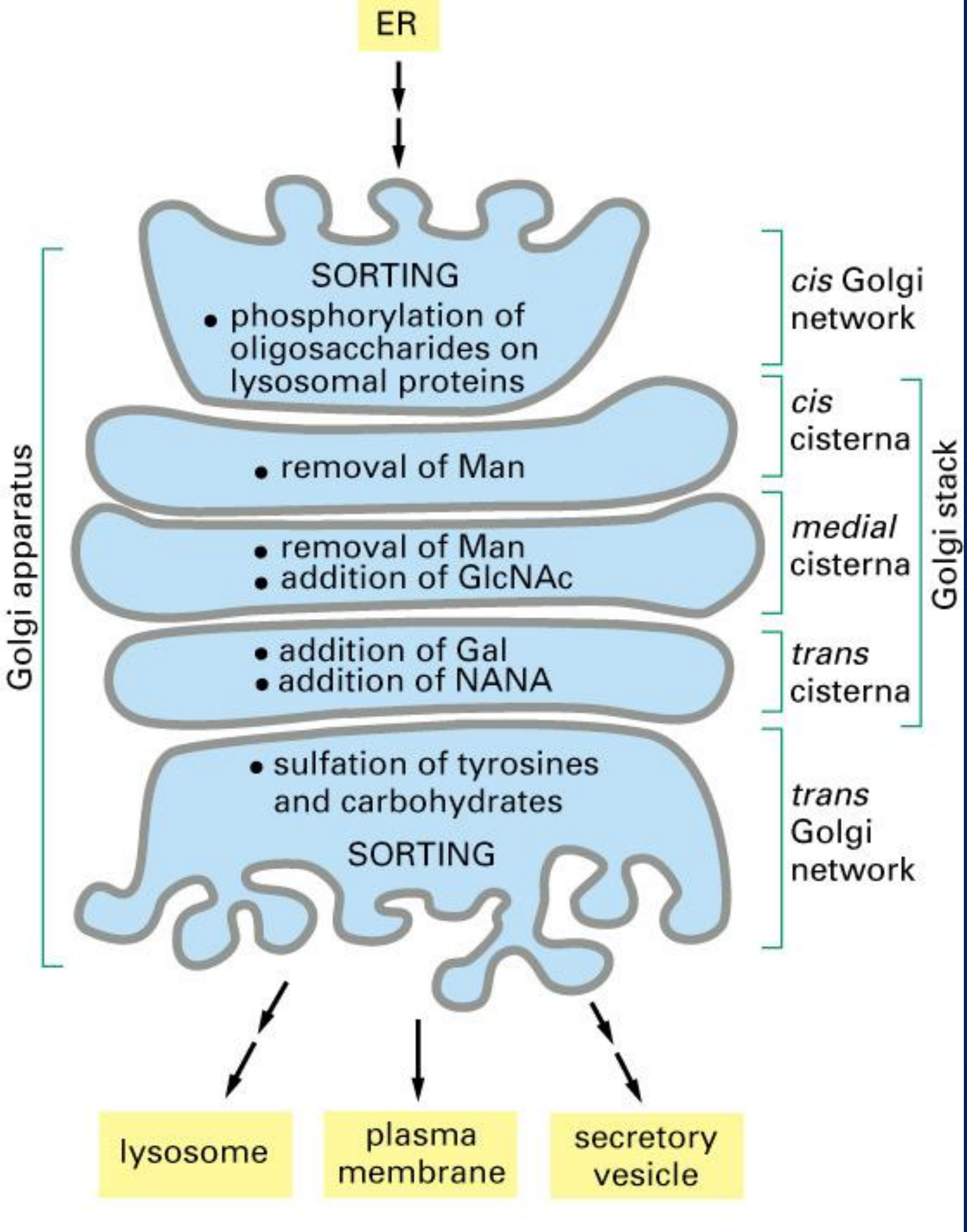
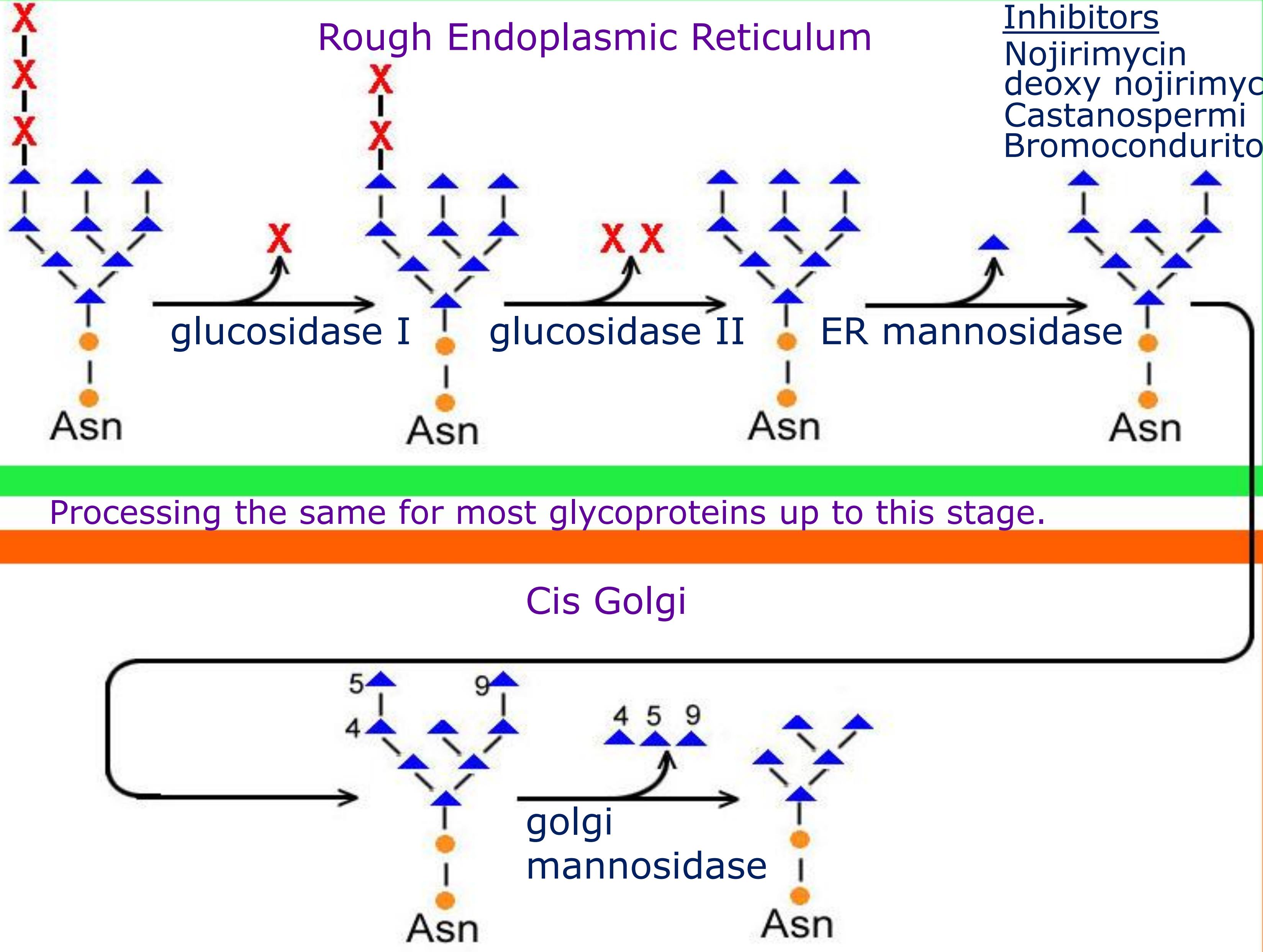


Figure 13–29. Molecular Biology of the Cell, 4th Edition.

# Rough Endoplasmic Reticulum



Inhibitors
Nojirimycin
deoxy nojirimycin
Castanospermi
Bromoconduritol

glucosidase I

glucosidase II

ER mannosidase

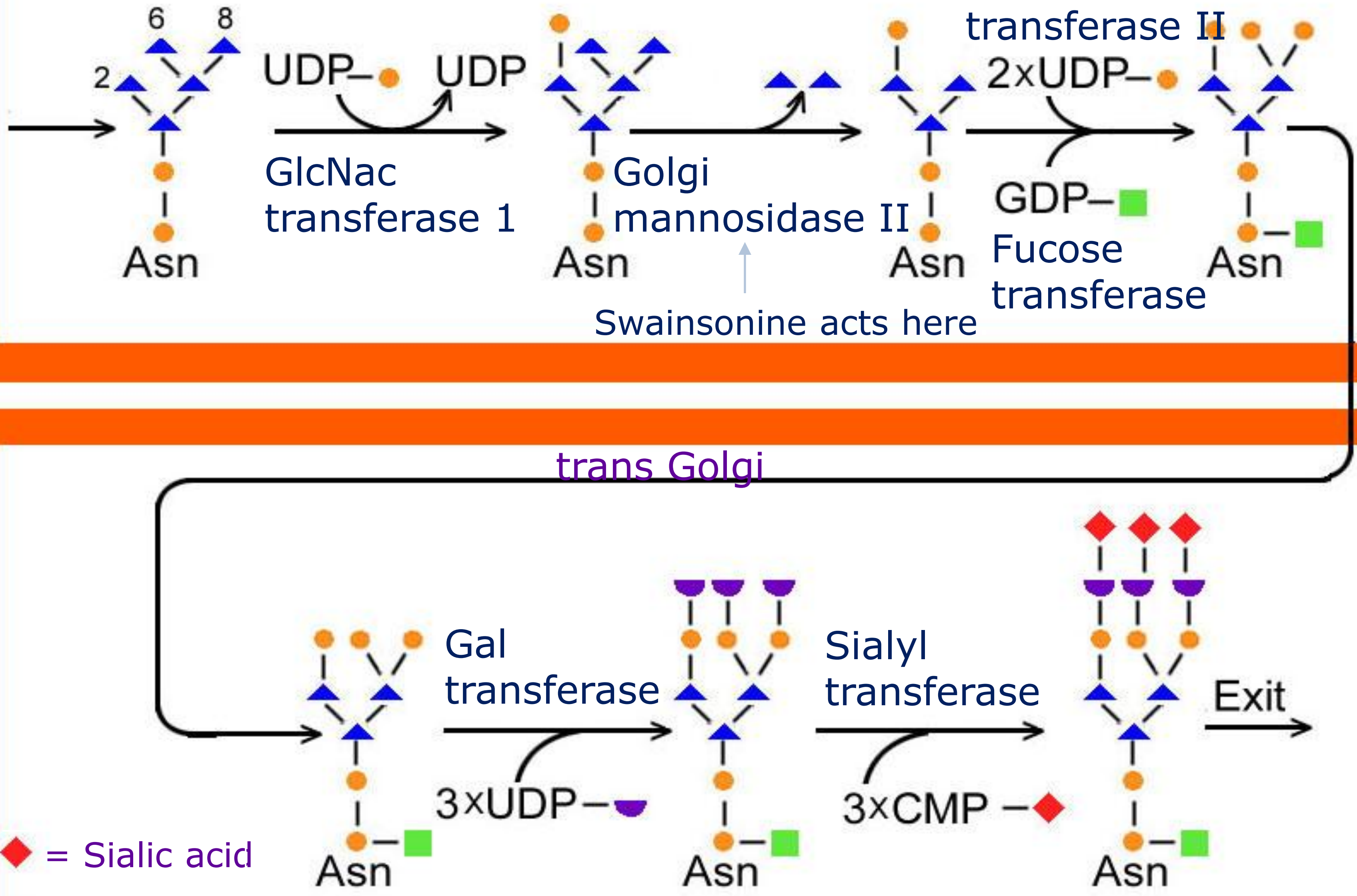
Processing the same for most glycoproteins up to this stage.

## Cis Golgi

golgi mannosidase

🟩 = L Fucose

medial Golgi



GlcNac transferase 1

Golgi mannosidase II

Swainsonine acts here

GlcNac transferase II

Fucose transferase

trans Golgi

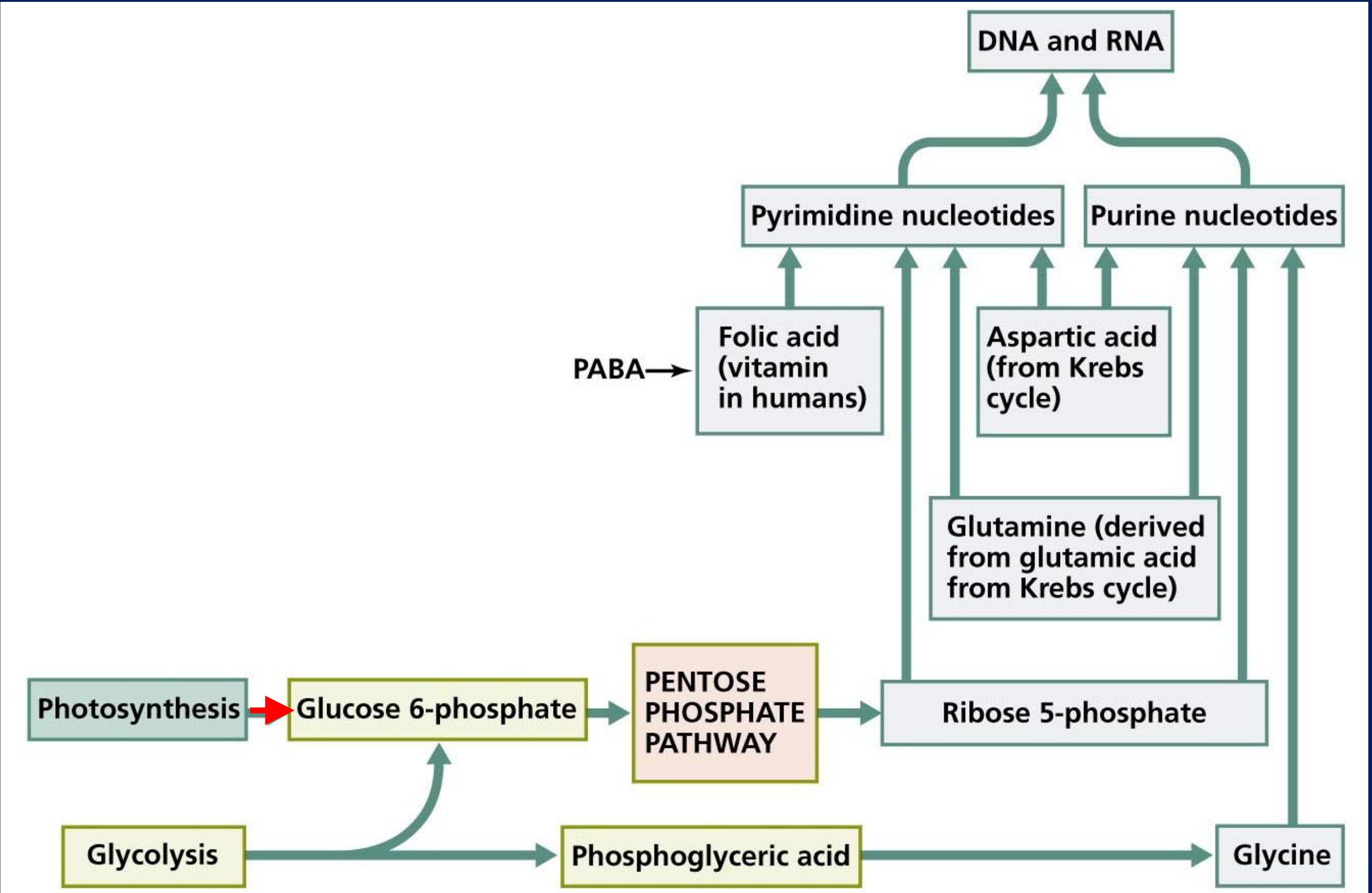
Gal transferase

Sialyl transferase

◆ = Sialic acid

= Galactose

# Anabolic Pathways – Nucleotide Biosynthesis



- цитозоль

# Biosynthesis of purines and pyrimidines, monomers of nucleotides

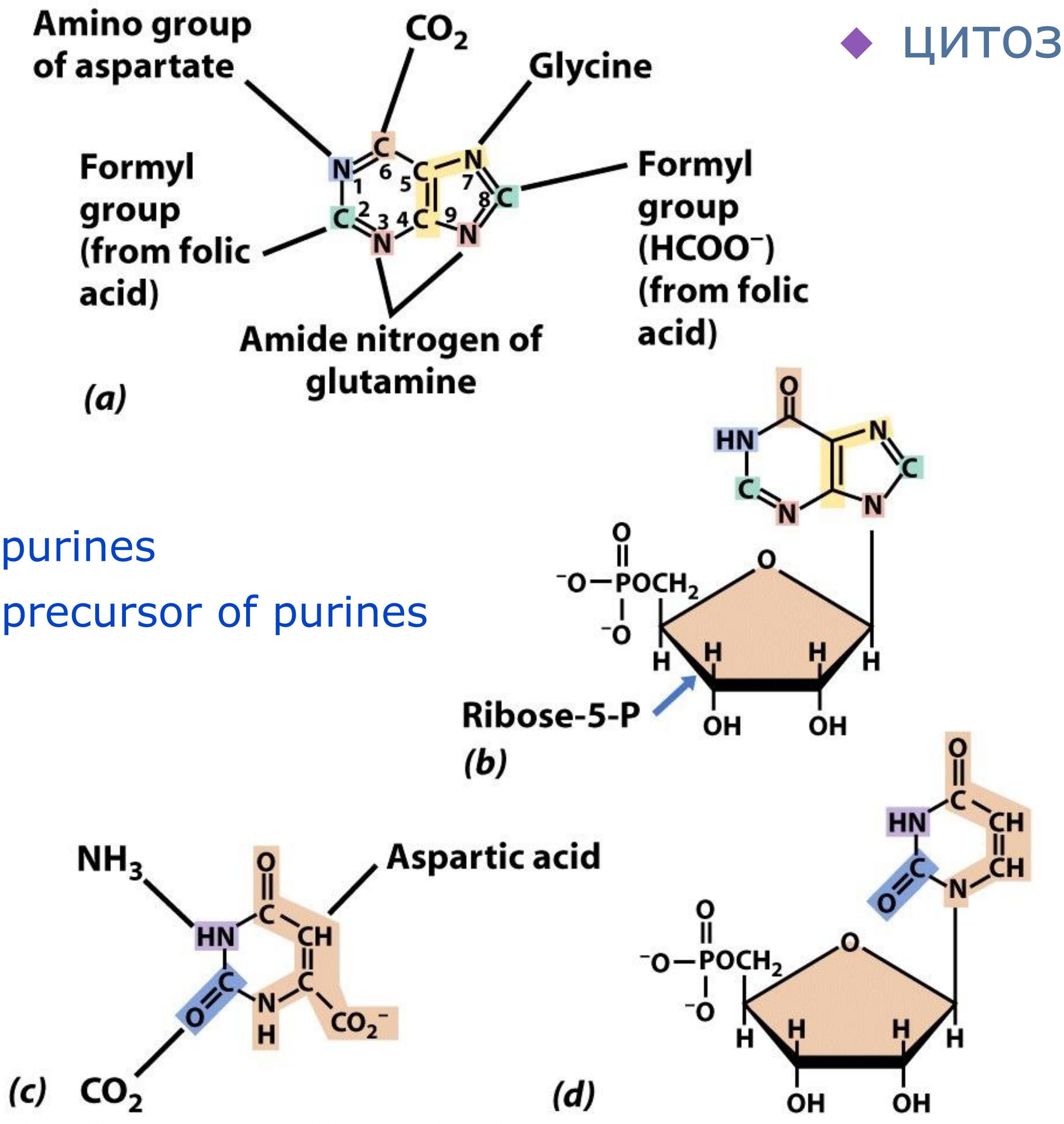


Figure 5-28 Brock Biology of Microorganisms 11/e
© 2006 Pearson Prentice Hall, Inc.

(a) Precursors of purines

(b) Inosinic acid, precursor of purines

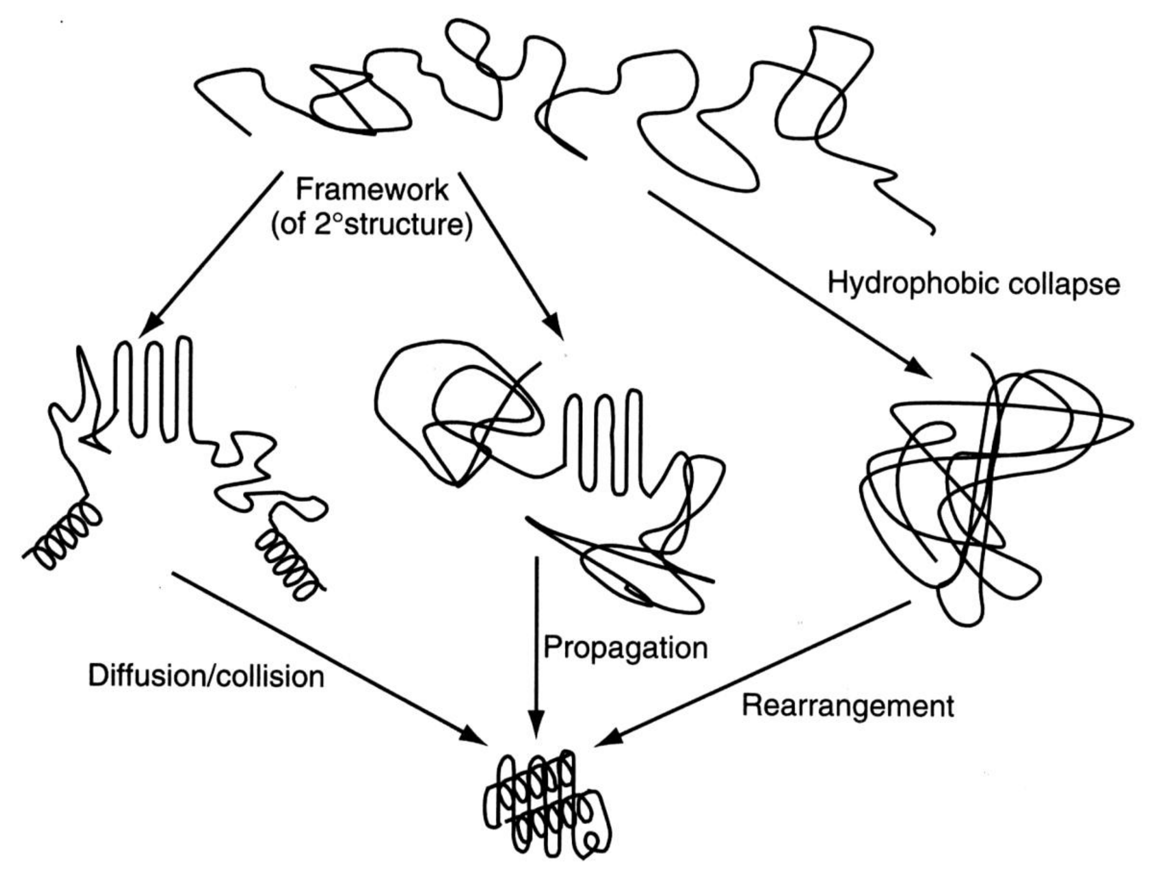
(c) Orotic acid and (d) uridylate are precursors of pyrimidines

# Синтез и фолдинг белков

- The folding of a protein should be phenomenally complex
  - For a 100 amino acid protein assuming 10 possible positions for an amino acid yeilds $10^{100}$ conformations. Sampled at a rate of 100 femtoseconds per confirmation It takes $10^{85}$ s to sample all possible conformations or $2.2*10^{68}$ times the age of the universe $4.4x10^{17}$s.
- Actual time proteins take to fold $10^{-1}$-$10^{-3}$s

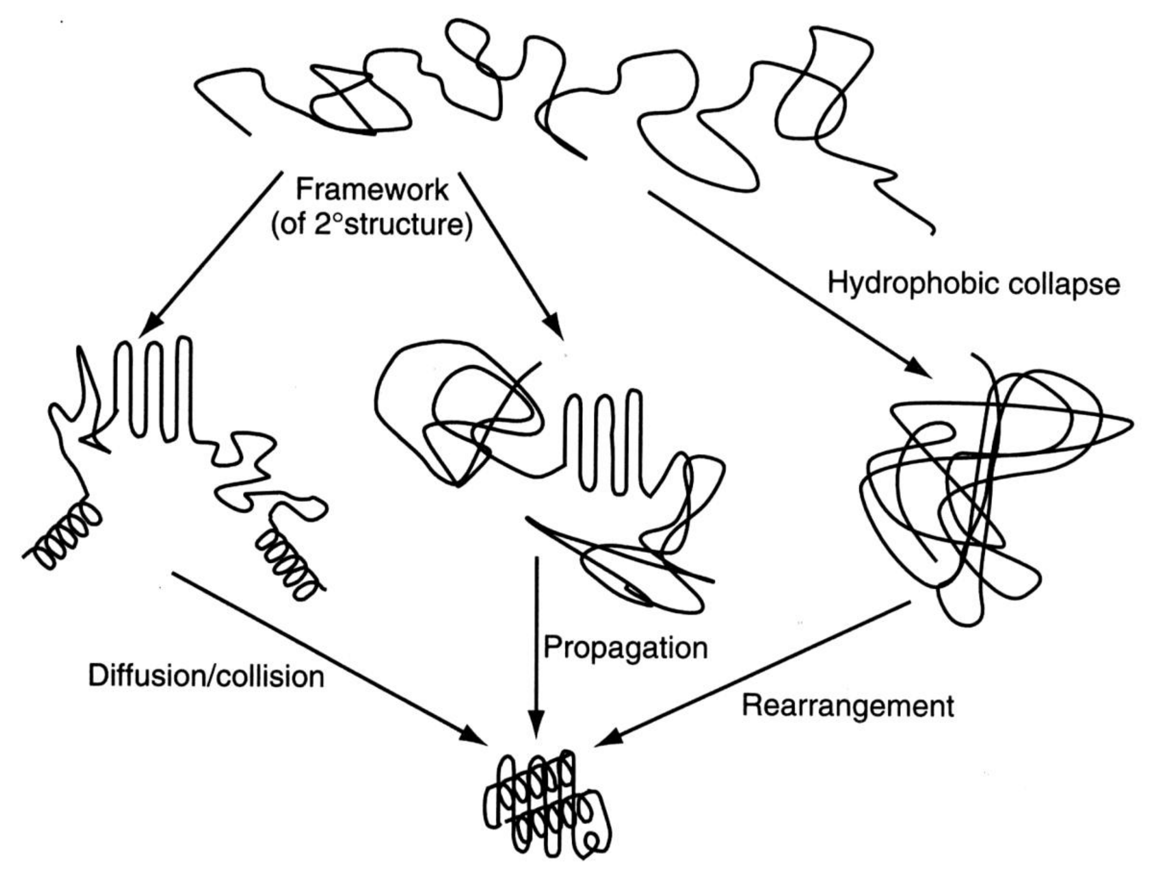
# Three Classic Models of Protein Folding

- The Framework model proposed that local elements of native local secondary structure could form independently of tertiary structure (Kim and Baldwin). These elements would diffuse until they collided, successfully adhering and coalescing to give the tertiary structure (diffusion-collision model)(Karplus & Weaver).



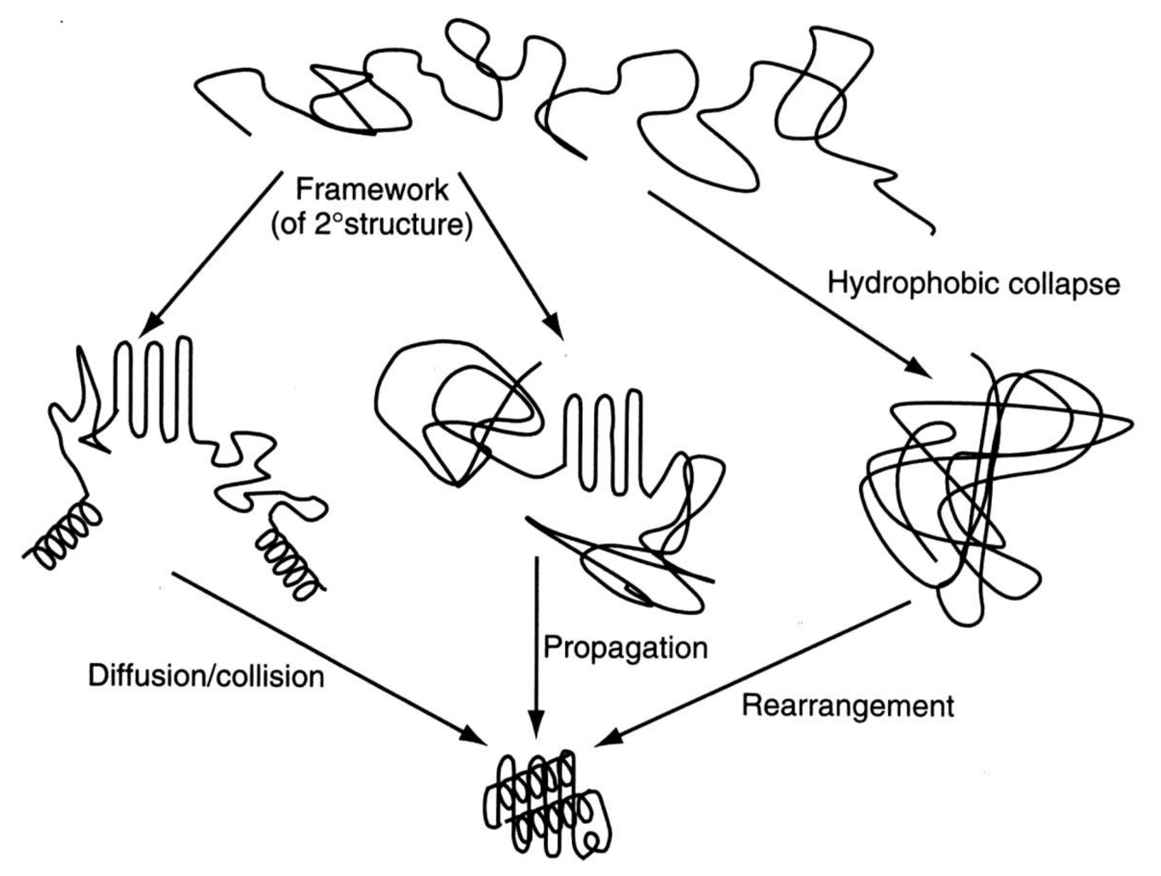
# The classic Nucleation Model

- The classic nucleation model postulated that some neighboring residues in the sequence would form native secondary structure that would act as a nucleus from which the native structure would propagate, in a stepwise manner. Thus, the tertiary structure would form as a necessary consequence of the secondary structure (Wetlaufer).

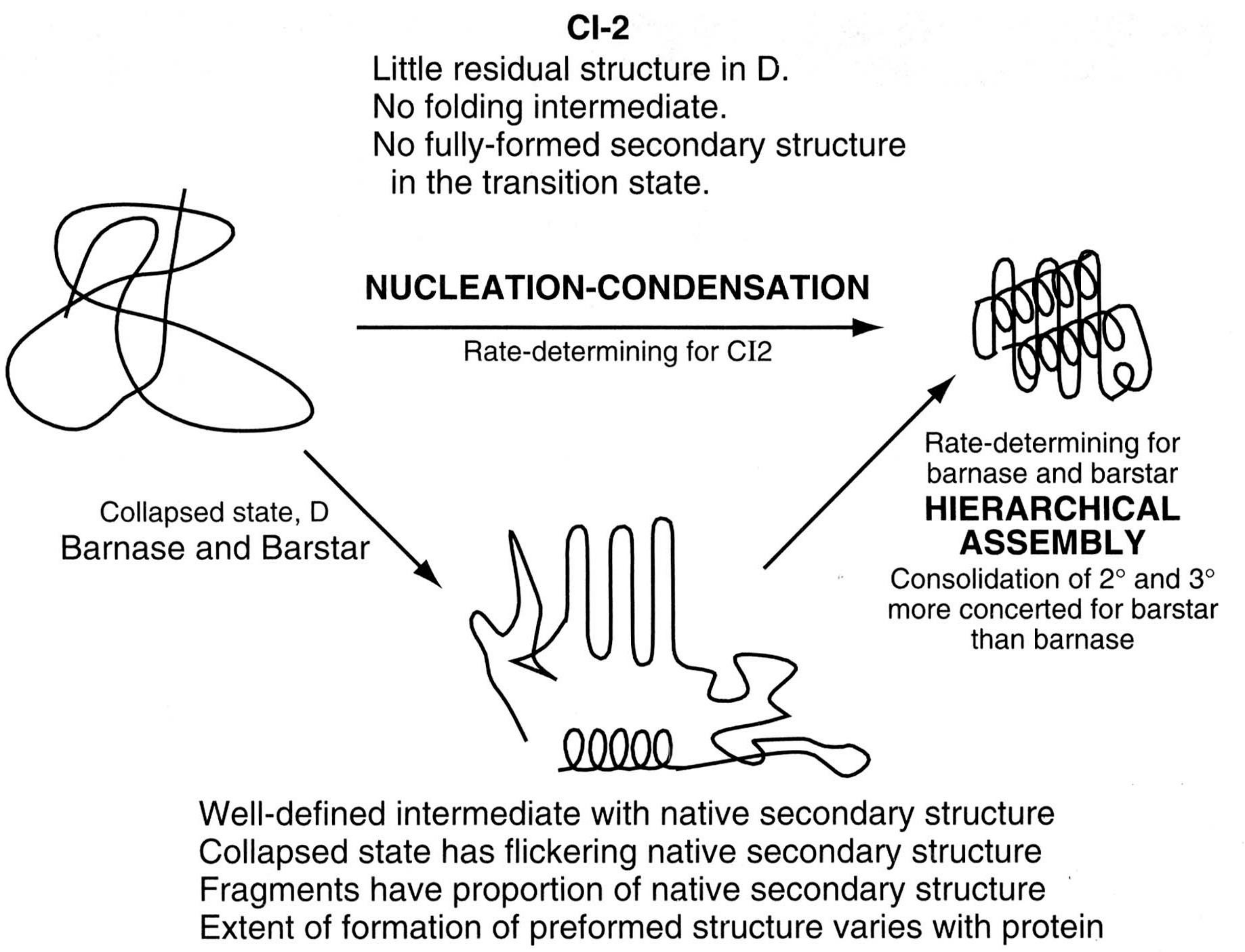


# The hydrophobic-collapse Model

The hydrophobic-collapse Model hypothesized that a protein would collapse rapidly around its hydrophobic sidechains and then rearrange from restricted conformational space occupied by the intermediate.  Here the secondary structure would be directed by native-like tertiary structure (Ptitsyn & Kuwajima).



# Unified Nucleation-condensation Scheme



**CI-2**

Little residual structure in D.
No folding intermediate.
No fully-formed secondary structure
in the transition state.

**NUCLEATION-CONDENSATION**

Rate-determining for CI2

Collapsed state, D
Barnase and Barstar

Rate-determining for
barnase and barstar

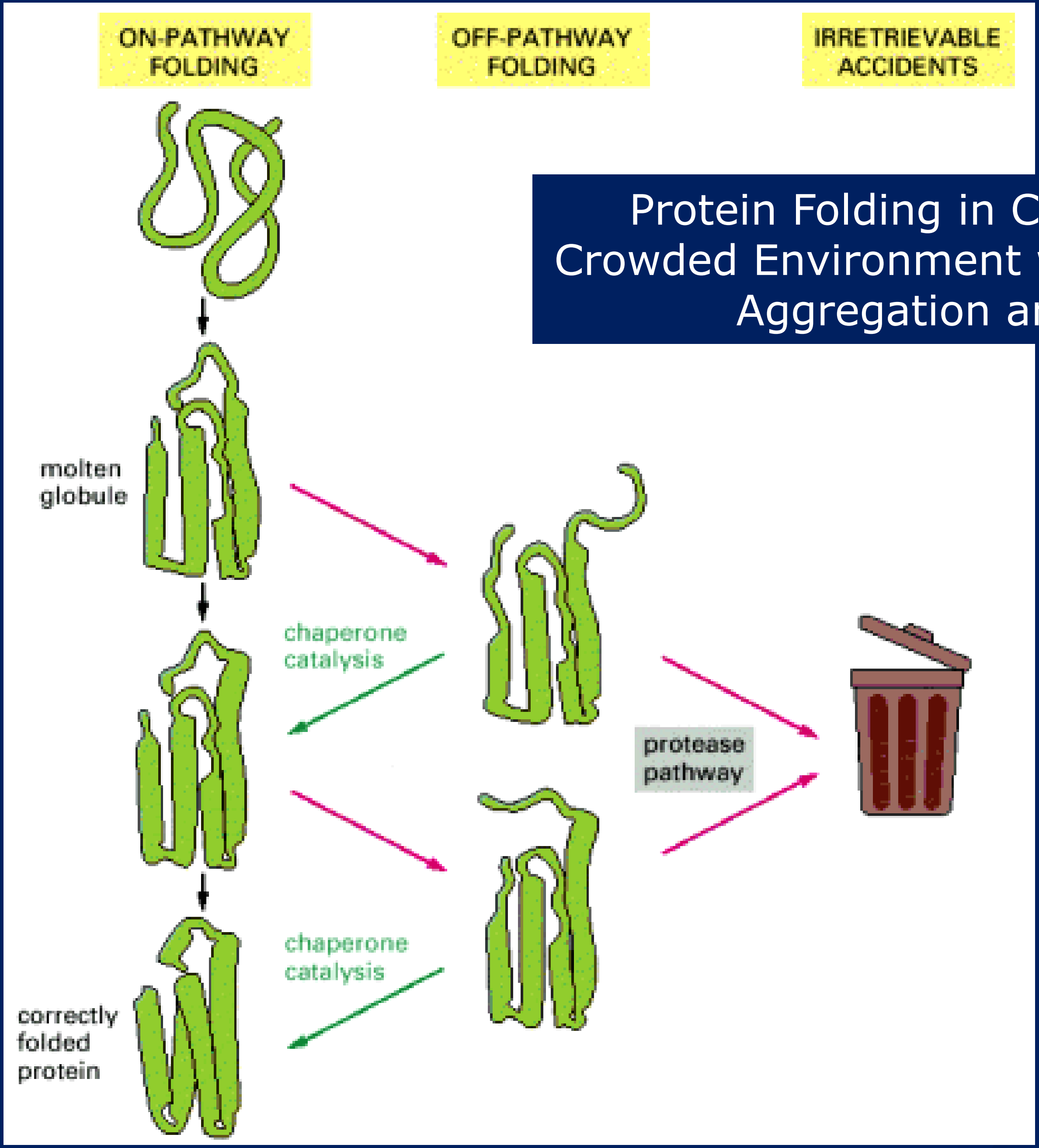
**HIERARCHICAL ASSEMBLY**

Consolidation of 2° and 3°
more concerted for barstar
than barnase

Well-defined intermediate with native secondary structure
Collapsed state has flickering native secondary structure
Fragments have proportion of native secondary structure
Extent of formation of preformed structure varies with protein

- It is unlikely that there is a single mechanism for protein folding.

# Protein Folding in Cells Occurs in a Crowded Environment where Misfolding & Aggregation are Favored



# Protein Folding Related Diseases

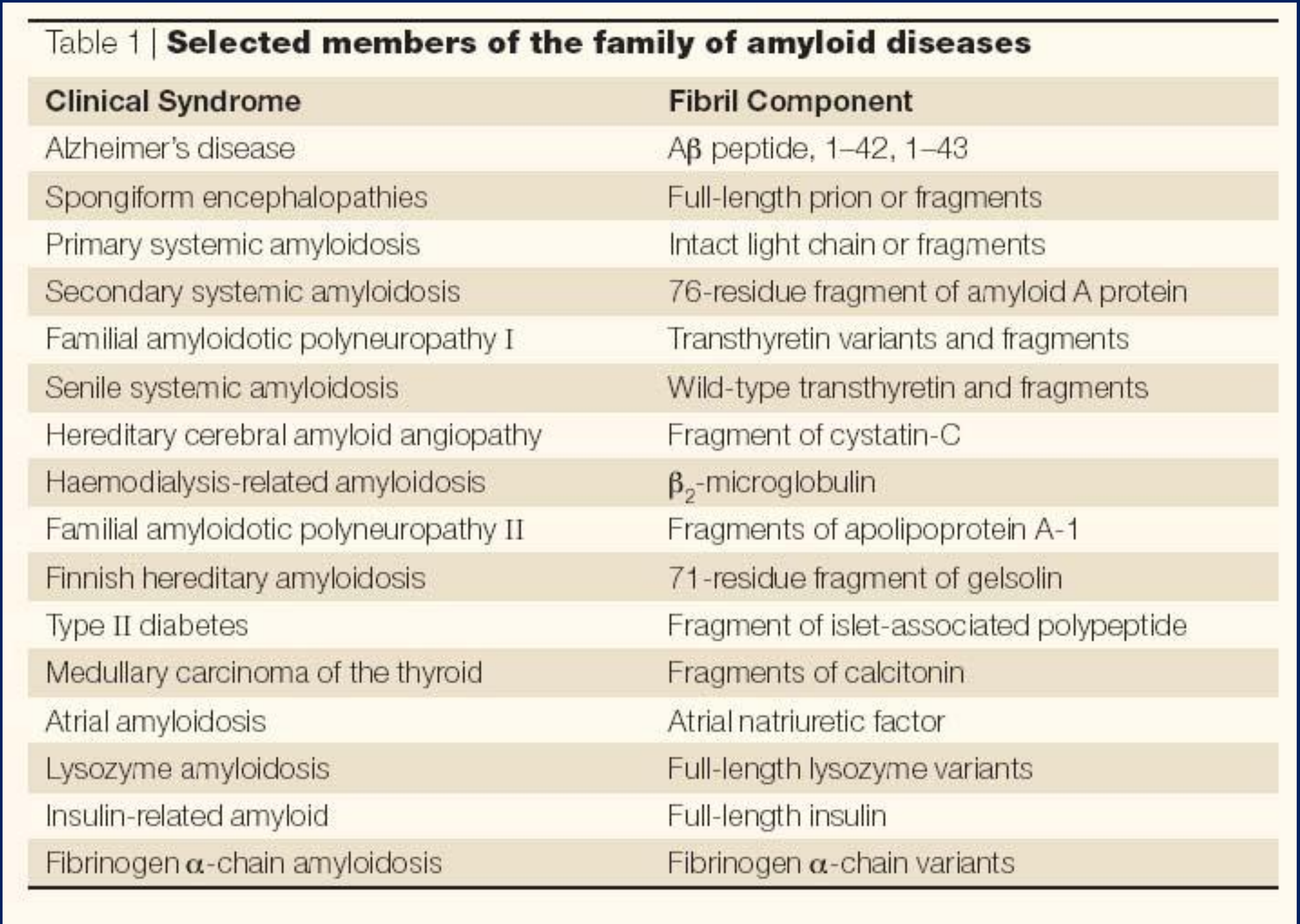


Table 1 | **Selected members of the family of amyloid diseases**

| Clinical Syndrome | Fibril Component |
|---|---|
| Alzheimer's disease | Aβ peptide, 1–42, 1–43 |
| Spongiform encephalopathies | Full-length prion or fragments |
| Primary systemic amyloidosis | Intact light chain or fragments |
| Secondary systemic amyloidosis | 76-residue fragment of amyloid A protein |
| Familial amyloidotic polyneuropathy I | Transthyretin variants and fragments |
| Senile systemic amyloidosis | Wild-type transthyretin and fragments |
| Hereditary cerebral amyloid angiopathy | Fragment of cystatin-C |
| Haemodialysis-related amyloidosis | $\beta_2$-microglobulin |
| Familial amyloidotic polyneuropathy II | Fragments of apolipoprotein A-1 |
| Finnish hereditary amyloidosis | 71-residue fragment of gelsolin |
| Type II diabetes | Fragment of islet-associated polypeptide |
| Medullary carcinoma of the thyroid | Fragments of calcitonin |
| Atrial amyloidosis | Atrial natriuretic factor |
| Lysozyme amyloidosis | Full-length lysozyme variants |
| Insulin-related amyloid | Full-length insulin |
| Fibrinogen α-chain amyloidosis | Fibrinogen α-chain variants |

## Two Classes of chaperons

- Constitutive – the chaperonins (GroEL/ES)
- Induced by stress – the HSP

# FAMILIES OF MOLECULAR CHAPERONES

Small heat shock proteins (hsp25) [*holders*]
- protect against cellular stress
- prevent aggregation in the lens (cataract)

Hsp60 system (cpn60, GroEL) *ATPase [(un)folders*]
- protein folding

Hsp70 system (DnaK, BiP) *ATPase [(un)folders*]
- stabilization of extended chains
- membrane translocation
- regulation of the heat shock response

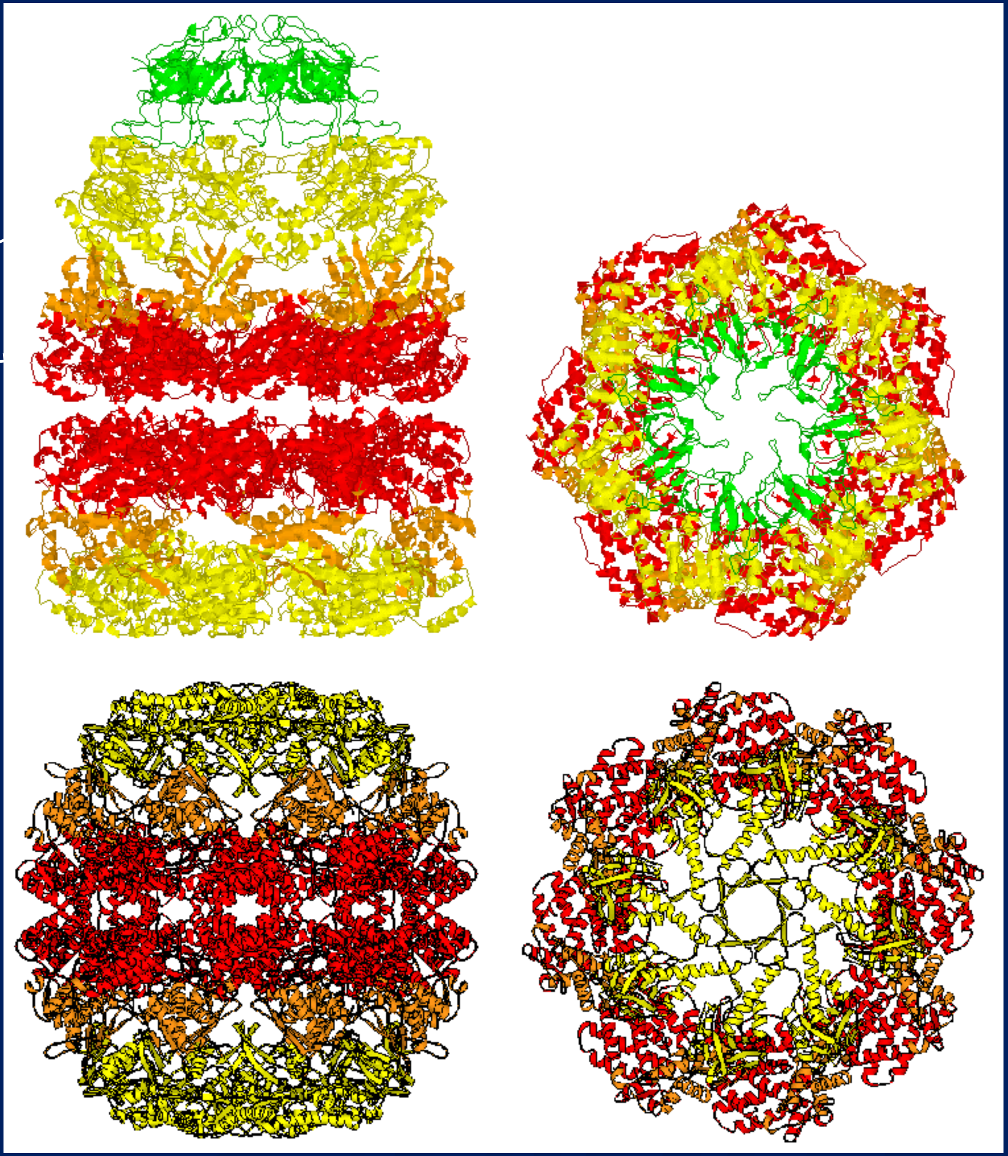
Hsp90 *ATPase* [*holder*]
- binding and stabilization/ regulation of steroid receptors, protein kinases

Hsp100 (Clp) *ATPase* [*unfolder*]
- thermotolerance, proteolysis, resolubilization of aggregates

Calnexin, calreticulin
- glycoprotein maturation in the ER
- quality control

Folding catalysts: PDI, PPI [*folders*]

# Bacterial Folding Complexes



GroES

E. coli
Gro EL Apical
Equatorial

The thermosome
from archaebacteria

# Properties of Chaperones

- Molecular chaperones interact with *unfolded or partially folded* protein subunits, e.g. nascent chains emerging from the ribosome, or extended chains being translocated across subcellular membranes.
- They stabilize non-native conformation and facilitate correct folding of protein subunits.
- They do not interact with native proteins, nor do they form part of the final folded structures. They also do not bind natively unfolded proteins.
- Some chaperones are non-specific, and interact with a wide variety of polypeptide chains, but others are restricted to specific targets.
- They often couple ATP binding/hydrolysis to the folding process.
- Essential for viability, their expression is often increased by cellular stress.
- **Main role:** They prevent inappropriate association or aggregation of exposed hydrophobic surfaces and direct their substrates into productive folding, transport or degradation pathways.

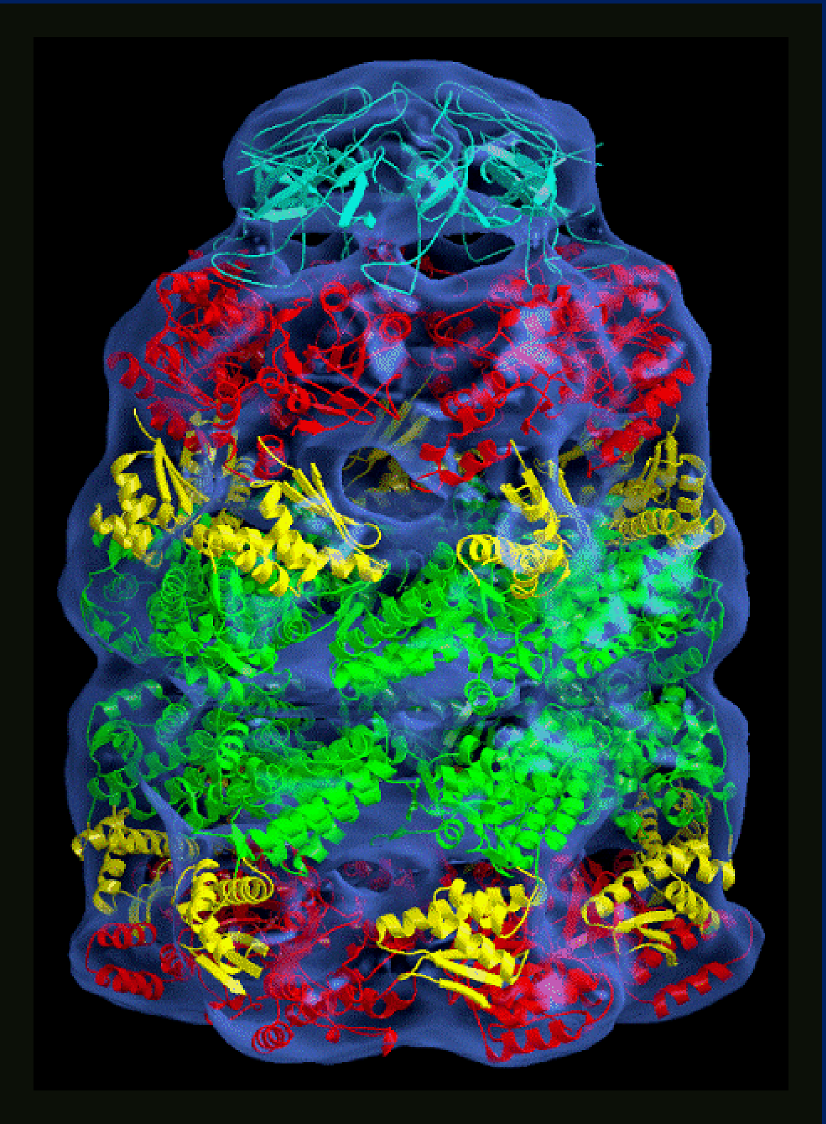
# Mechanism of Action of Chaperones

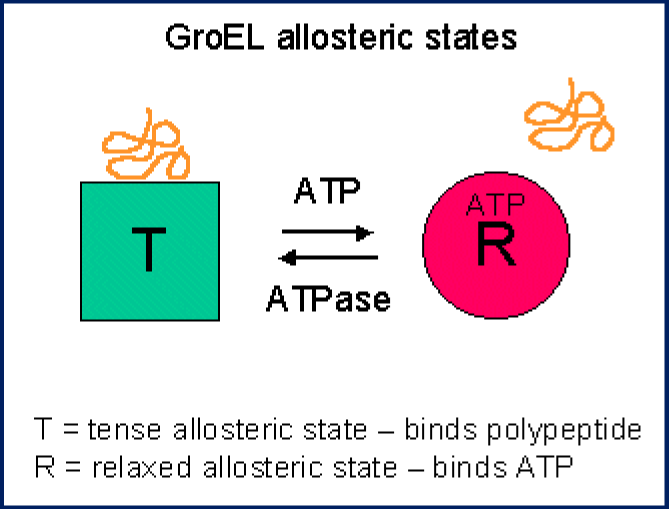
The precise mechanism is not yet known and may vary with the substrate. There are **two general ideas**.

1. The chaperone binds to the unfolded form of the protein and sequesters it within the central cavity. There it undergoes *repeated cycles of binding and unfolding* until the native form is reached.

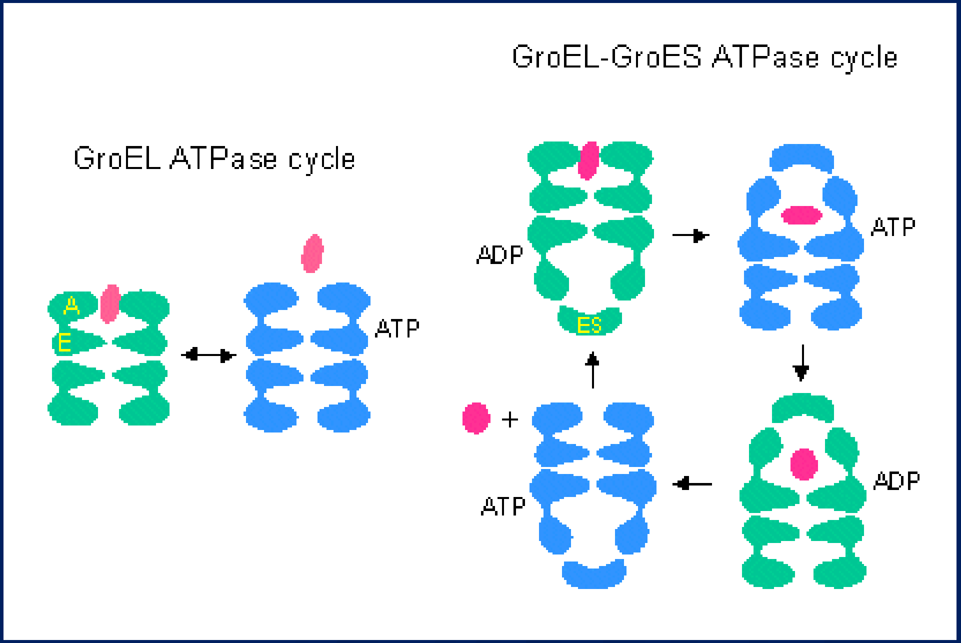
2. The chaperone binds to the unfolded or misfolded form of the protein transiently and acts to further unfold it. The protein is never held within the central cavity. The protein *is continuously unfolded and allowed to restart folding* until it attains a native fold.

# The conformational cycle of GroEL/ES



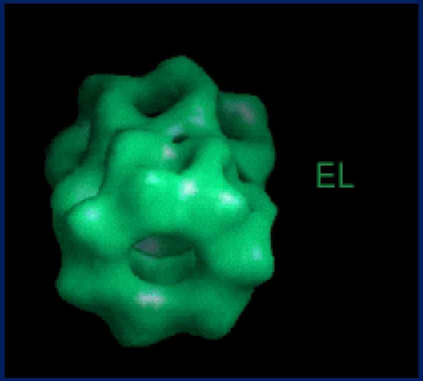

GroEL allosteric states

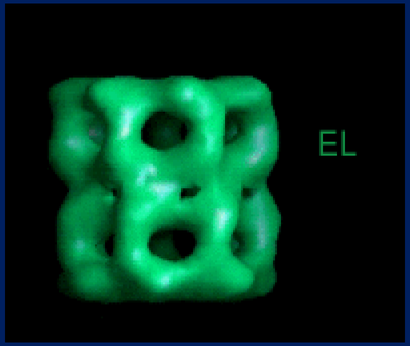
T = tense allosteric state – binds polypeptide
R = relaxed allosteric state – binds ATP

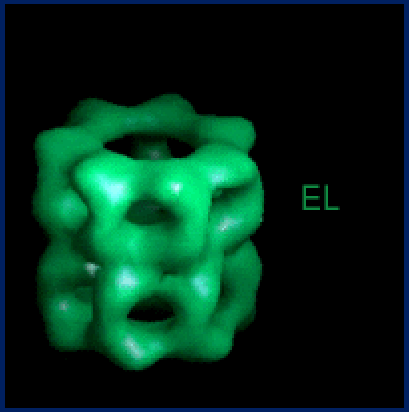

GroEL-GroES ATPase cycle

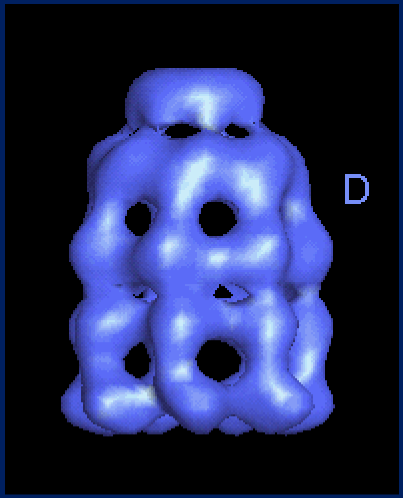
GroEL ATPase cycle

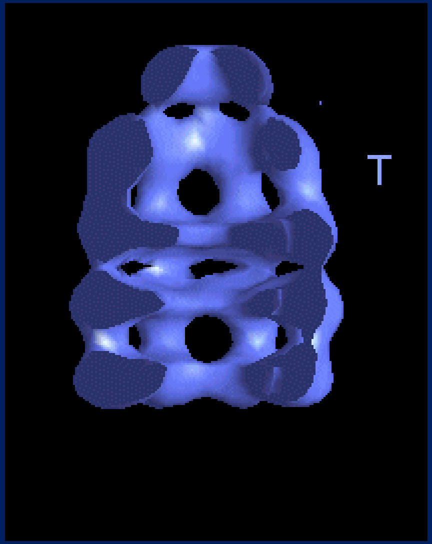
# GroEL-ES in action

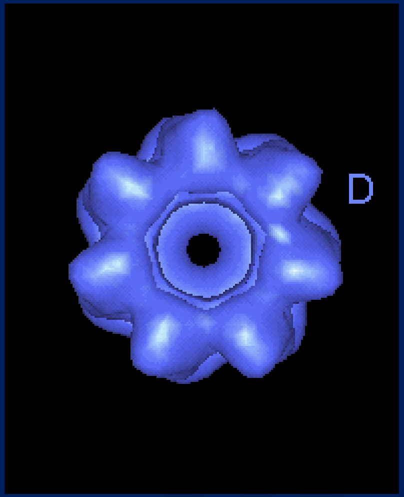






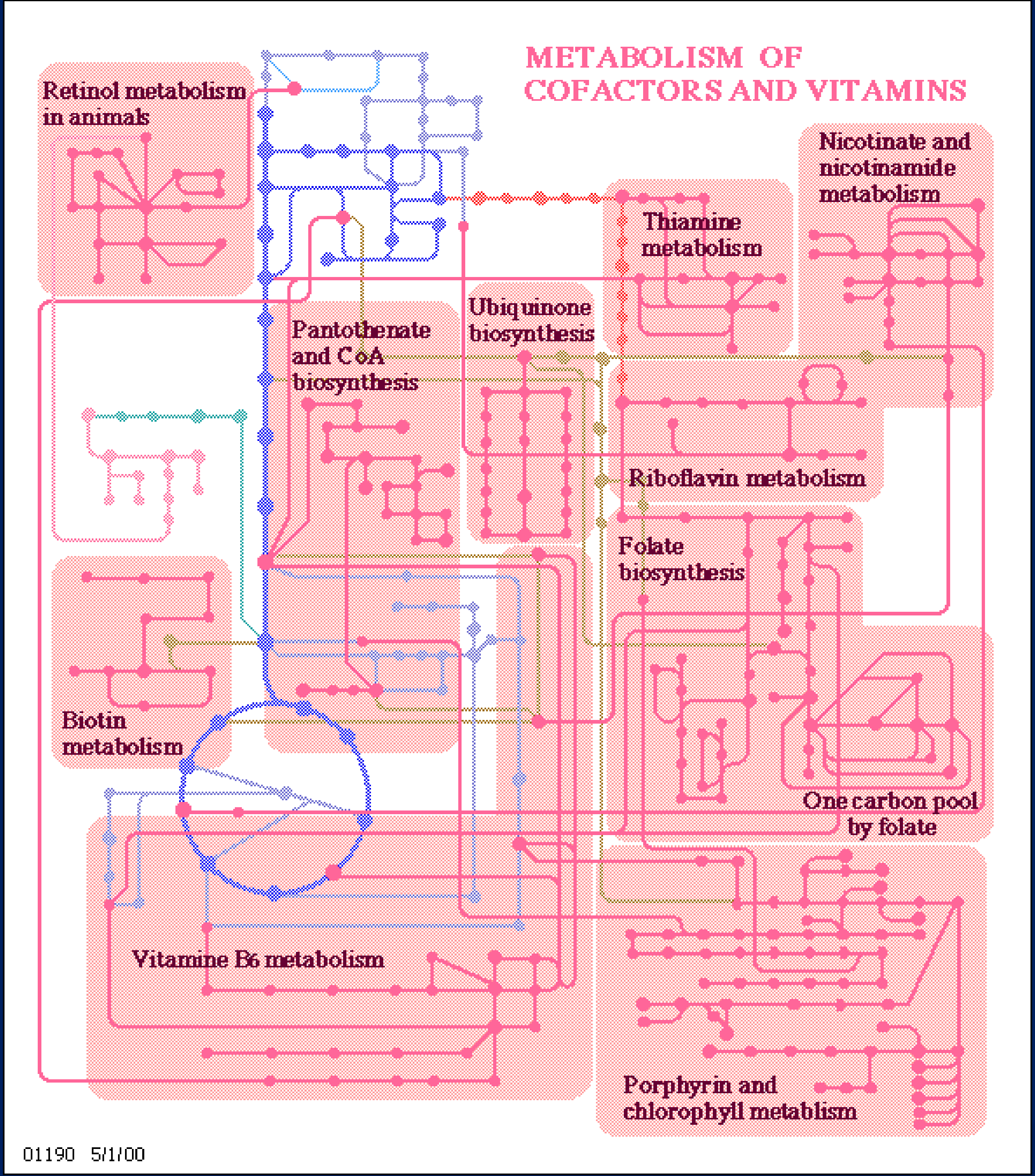




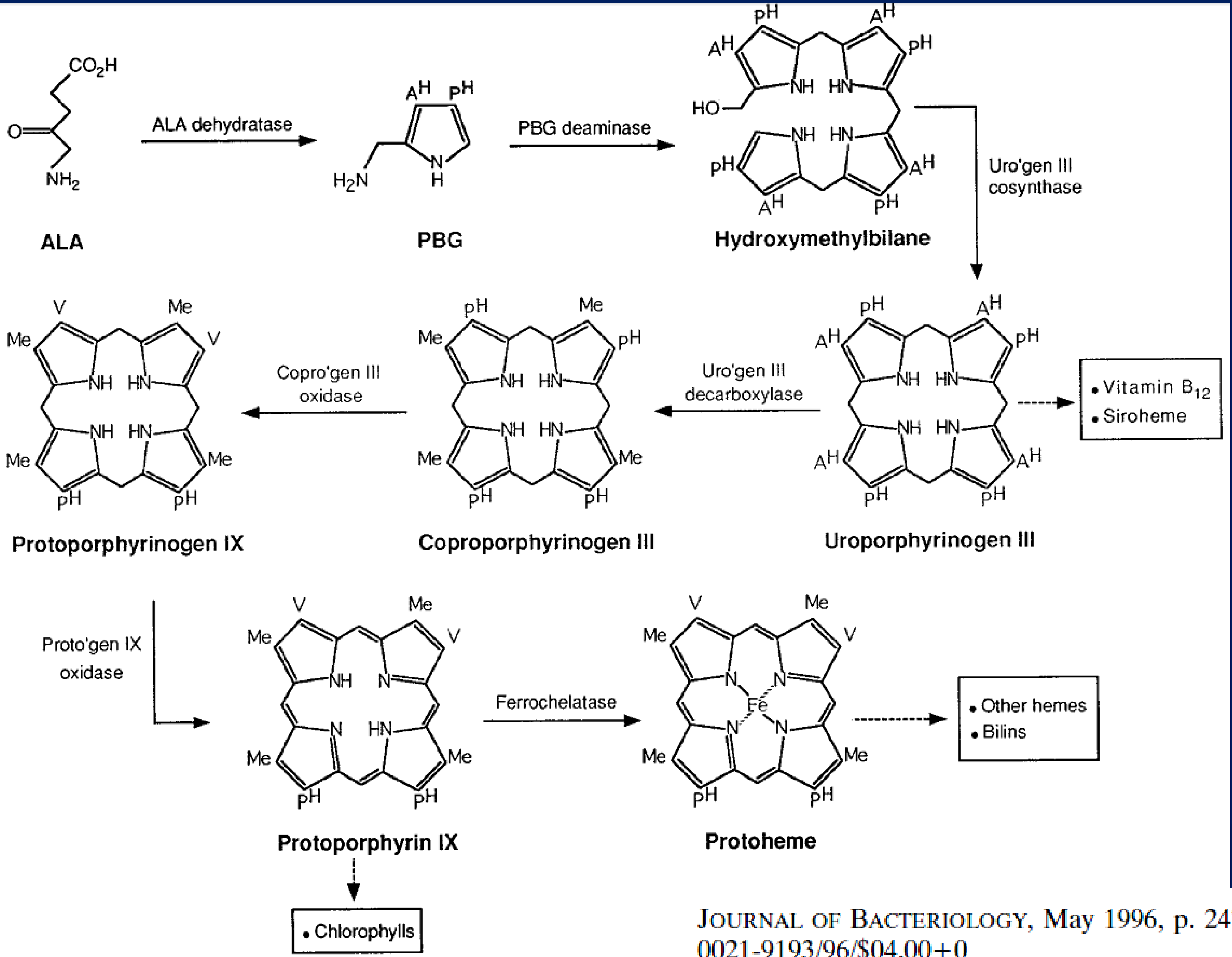


# Синтезы коферментов

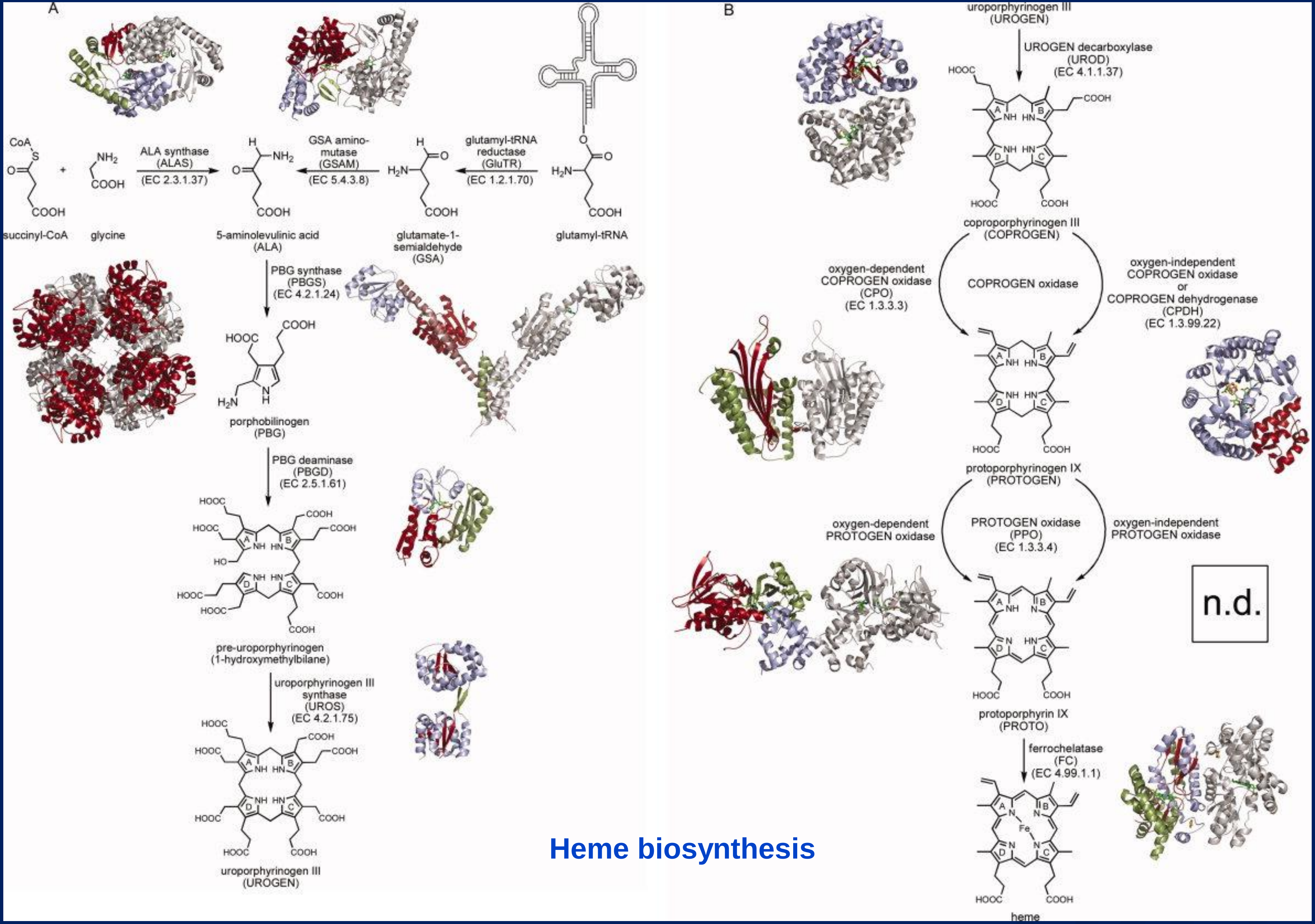
- Синтез гема
- Синтез флавина
- Синтез тетрагидрофолата
- Синтез пантотеновой кислоты и CoA
- Синтез SAM
- Синтез тиамина
- Синтез пиридоксаля
- Синтез биотина


METABOLISM OF COFACTORS AND VITAMINS
Retinol metabolism in animals
Nicotinate and nicotinamide metabolism
Thiamine metabolism
Ubiquinone biosynthesis
Pantothenate and CoA biosynthesis
Riboflavin metabolism
Folate biosynthesis
Biotin metabolism
One carbon pool by folate
Vitamine B6 metabolism
Porphyrin and chlorophyll metablism
01190 5/1/00

# Heme biosynthesis



JOURNAL OF BACTERIOLOGY, May 1996, p. 2471–2478
0021-9193/96/$04.00+0


A
CoA
S
NH$_2$
COOH
COOH
succinyl-CoA
glycine
ALA synthase (ALAS) (EC 2.3.1.37)
5-aminolevulinic acid (ALA)
GSA amino-mutase (GSAM) (EC 5.4.3.8)
glutamate-1-semialdehyde (GSA)
glutamyl-tRNA reductase (GluTR) (EC 1.2.1.70)
glutamyl-tRNA
PBG synthase (PBGS) (EC 4.2.1.24)
porphobilinogen (PBG)
PBG deaminase (PBGD) (EC 2.5.1.61)
pre-uroporphyrinogen (1-hydroxymethylbilane)
uroporphyrinogen III synthase (UROS) (EC 4.2.1.75)
uroporphyrinogen III (UROGEN)
B
uroporphyrinogen III (UROGEN)
UROGEN decarboxylase (UROD) (EC 4.1.1.37)
coproporphyrinogen III (COPROGEN)
oxygen-dependent COPROGEN oxidase (CPO) (EC 1.3.3.3)
COPROGEN oxidase
oxygen-independent COPROGEN oxidase or COPROGEN dehydrogenase (CPDH) (EC 1.3.99.22)
protoporphyrinogen IX (PROTOGEN)
oxygen-dependent PROTOGEN oxidase
PROTOGEN oxidase (PPO) (EC 1.3.3.4)
oxygen-independent PROTOGEN oxidase
n.d.
protoporphyrin IX (PROTO)
ferrochelatase (FC) (EC 4.99.1.1)
heme

## Heme biosynthesis

**Protein Science** Volume 19, Issue 6, pages 1137-1161, 27 APR 2010 DOI: 10.1002/pro.405
http://onlinelibrary.wiley.com/doi/10.1002/pro.405/full#fig1

# Heme biosynthesis

A: The first cyclic tetrapyrrole uroporphyrinogen III is formed from the precursor **5-aminolevulinic acid** in three enzymatic steps via the intermediates porphobilinogen and pre-uroporphyrinogen. Depending on the organism, ALA is either synthesized by

← condensation of glycine with succinyl-CoA or

← from tRNA-bound glutamate via glutamate-1-semialdehyde.

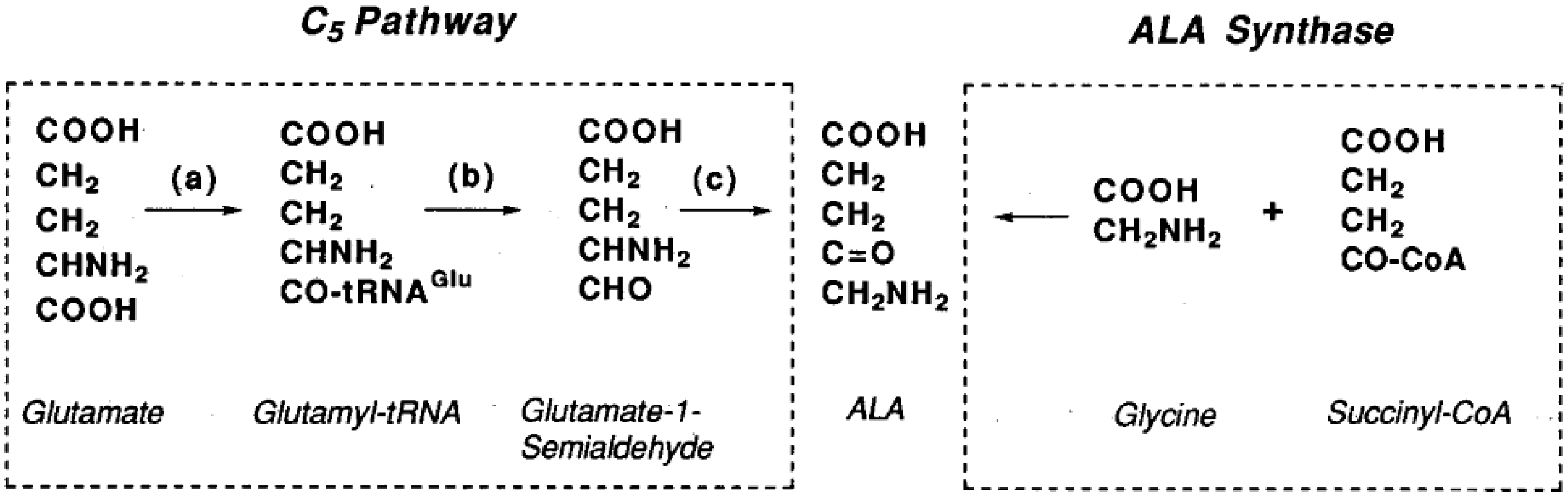
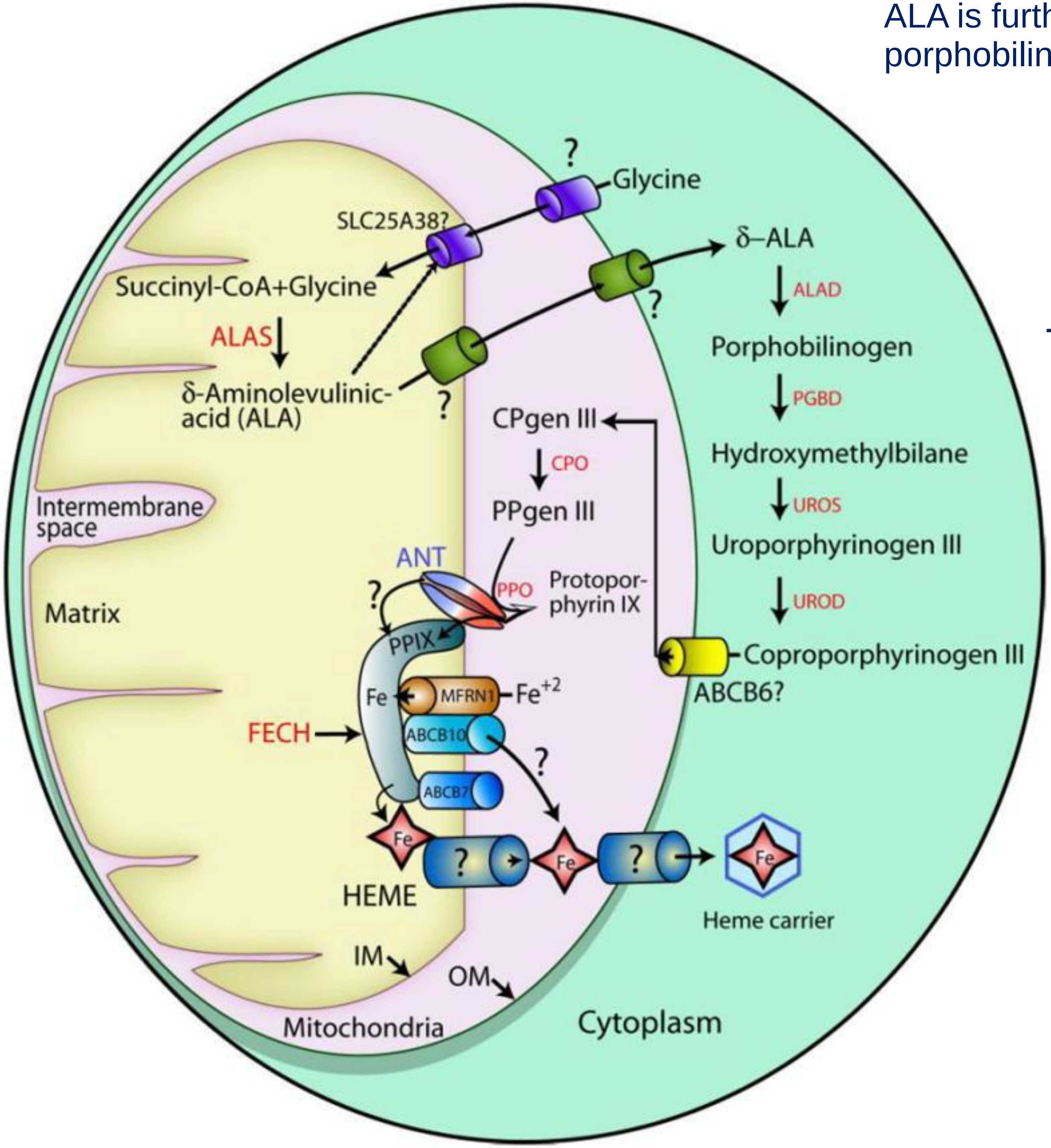
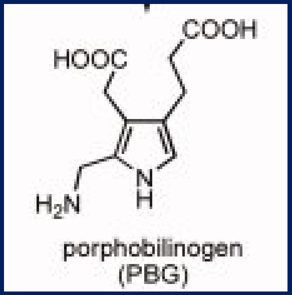


FIG. 2. ALA synthesis by ALA synthase or the $C_5$ pathway. The enzymes of the $C_5$ pathway and their cofactors are as follows: (a) glutamyl-tRNA synthesis (requires Mg-ATP); (b) glutamyl-tRNA reductase [pyridoxal phosphate dependent and requires NAD(P)H]; and (c) glutamate 1-semialdehyde aminotransferase.

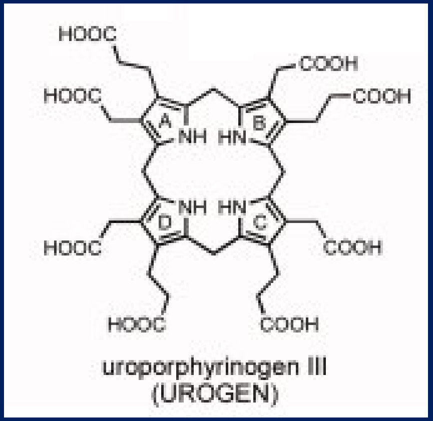
ALA is furth
porphobilin



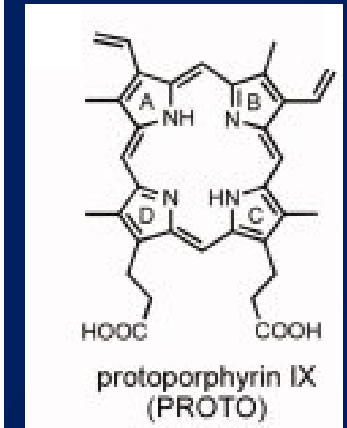


porphobilinogen
(PBG)

→

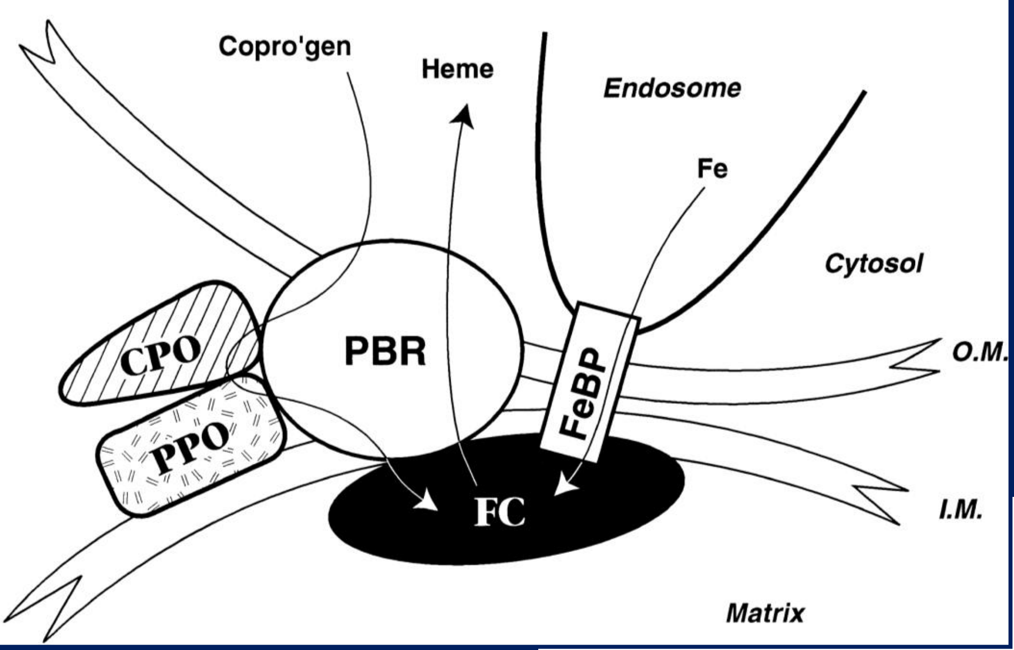


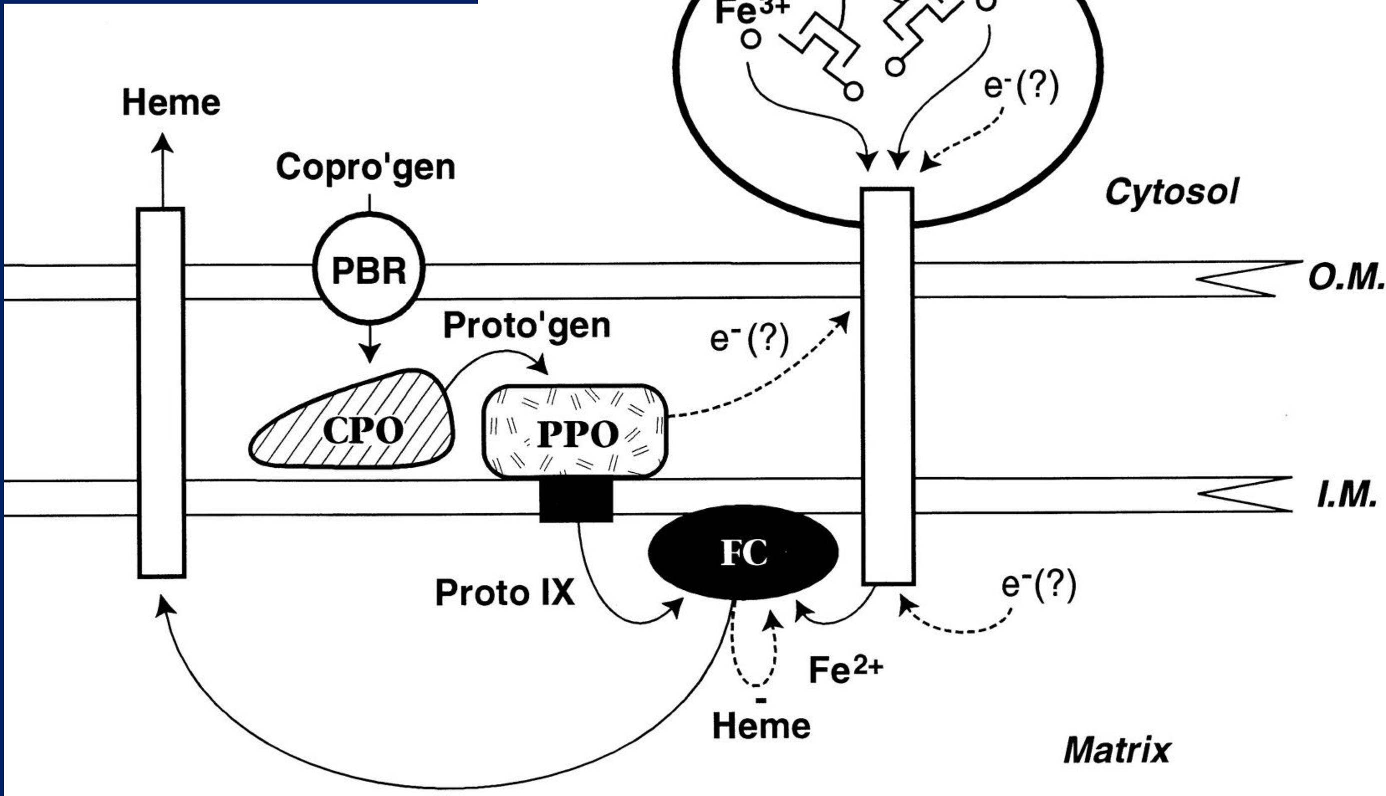
uroporphyrinogen III
(UROGEN)



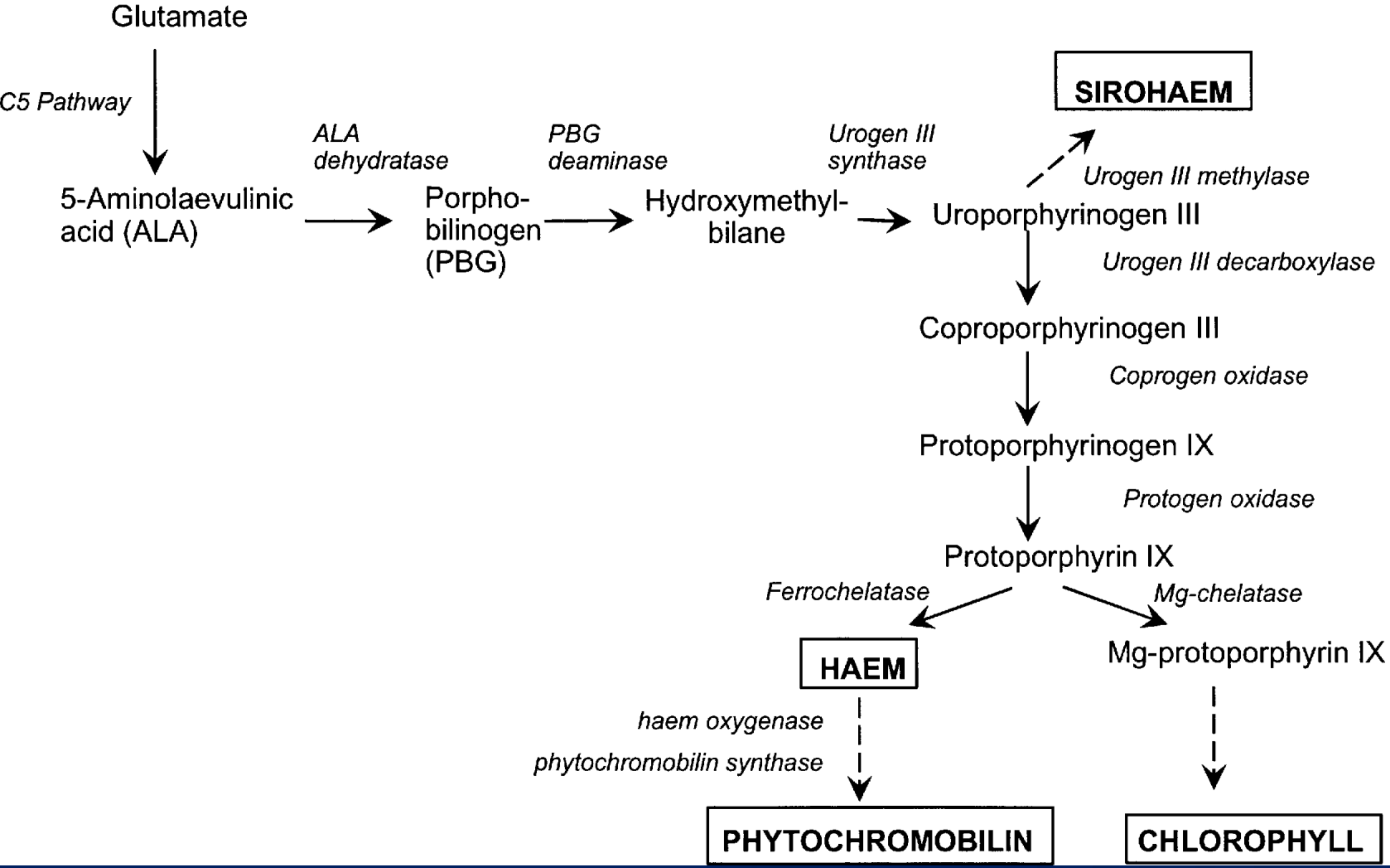
protoporphyrin IX
(PROTO)

# Ferrochelatase inserts $Fe^{2+}$





# Chlorophyll synthesis in plants



Glutamate

*C5 Pathway*

5-Aminolaevulinic acid (ALA)

*ALA dehydratase*

Porphobilinogen (PBG)

*PBG deaminase*

Hydroxymethylbilane

*Urogen III synthase*

Uroporphyrinogen III

*Urogen III methylase*

**SIROHAEM**

*Urogen III decarboxylase*

Coproporphyrinogen III

*Coprogen oxidase*

Protoporphyrinogen IX

*Protogen oxidase*

Protoporphyrin IX

*Ferrochelatase*

**HAEM**

*haem oxygenase*

*phytochromobilin synthase*

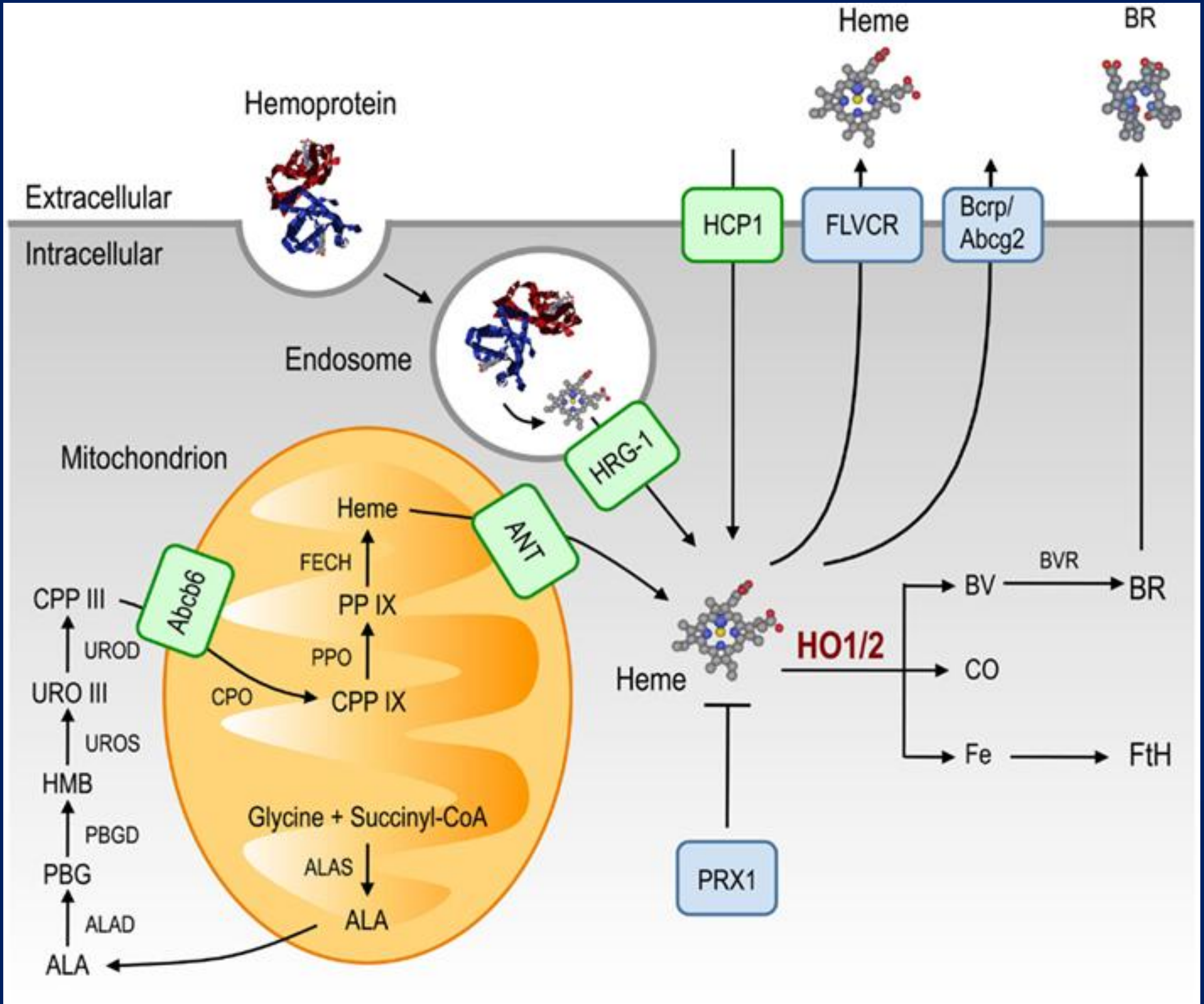
**PHYTOCHROMOBILIN**

*Mg-chelatase*

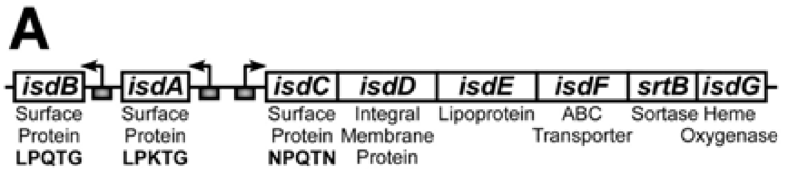
Mg-protoporphyrin IX

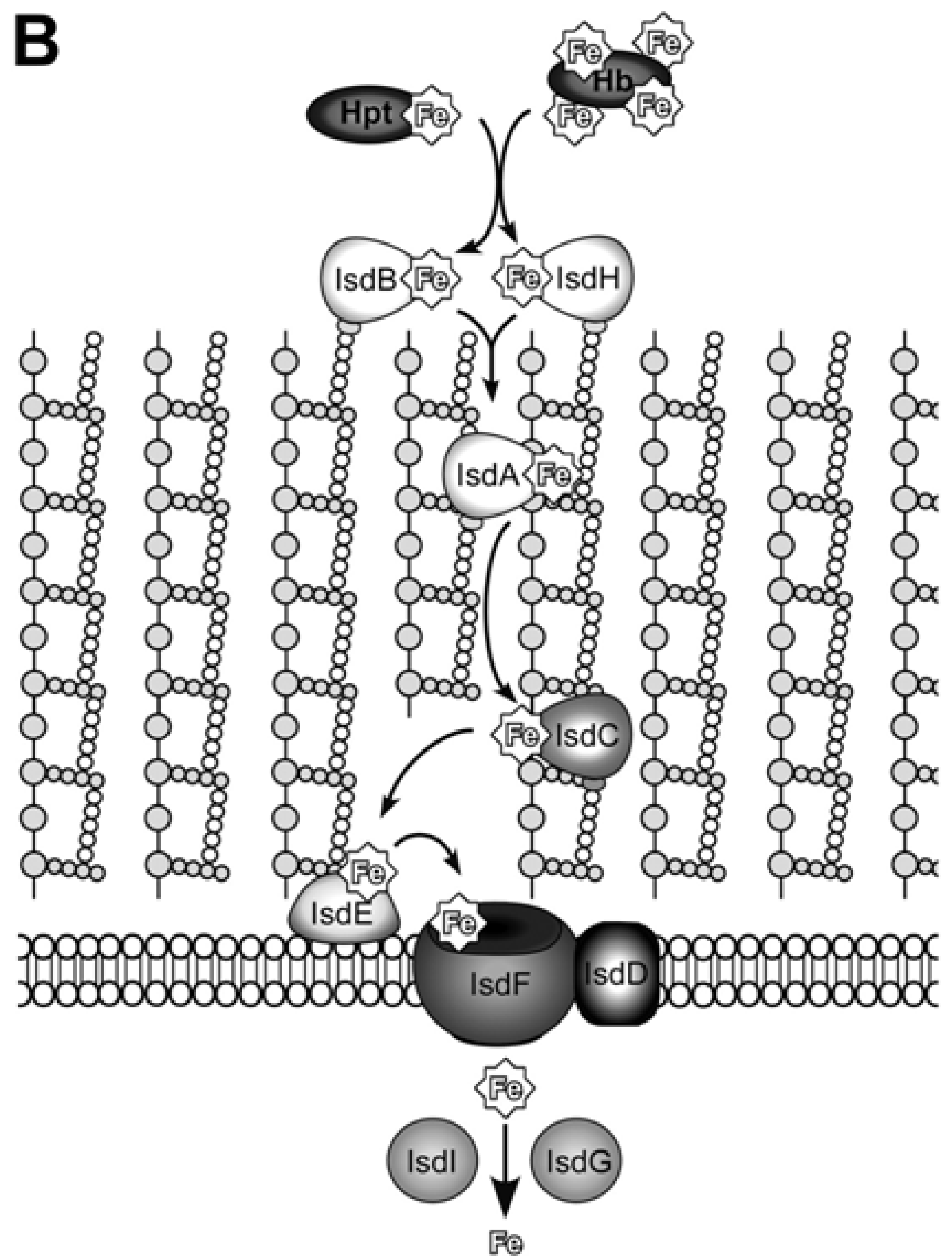
**CHLOROPHYLL**

# Heme transport



# Isd-mediated heme-iron uptake in S. aureus.





Marraffini L A et al. Microbiol. Mol. Biol. Rev. 2006;70:192-221

Microbiology and Molecular Biology Reviews

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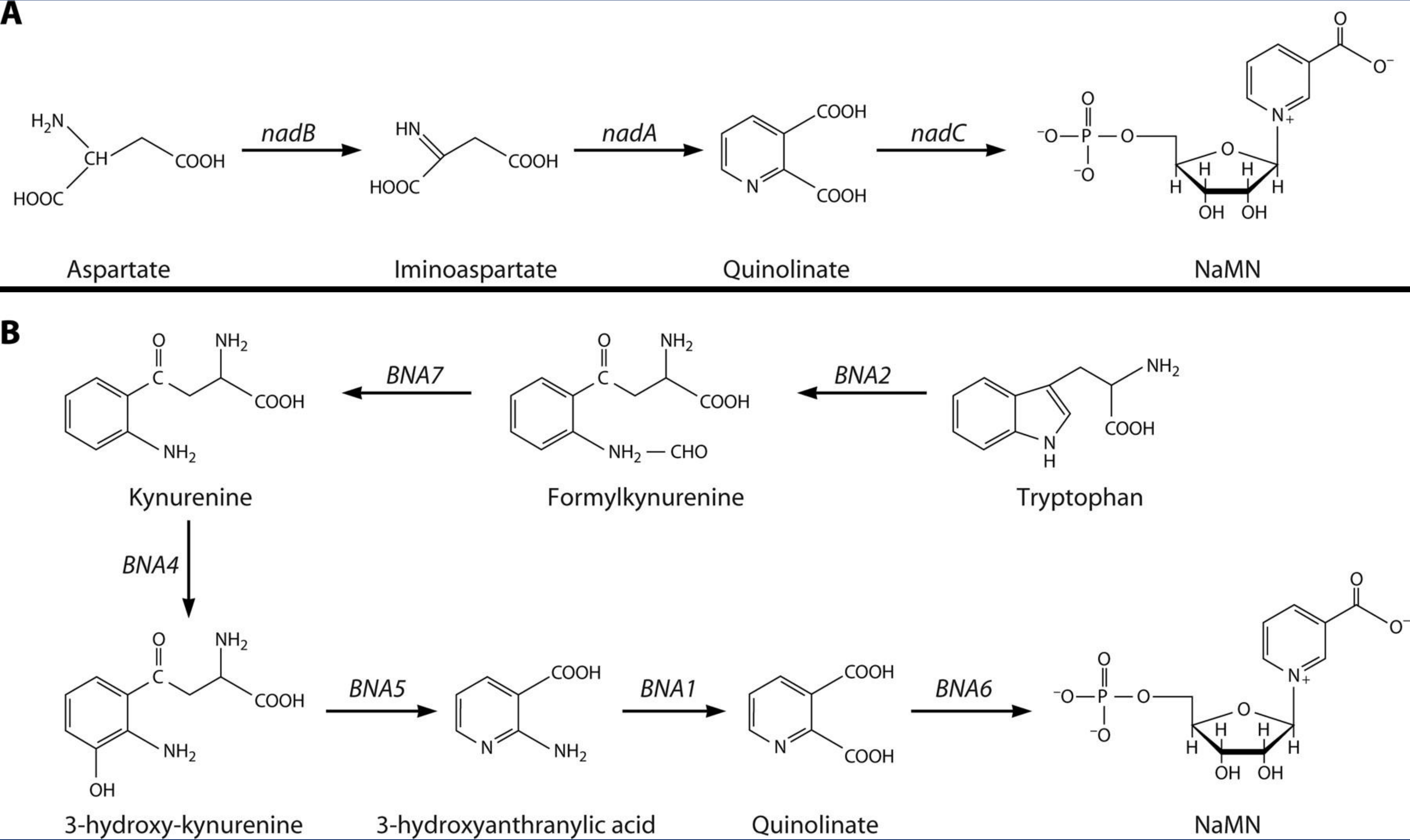
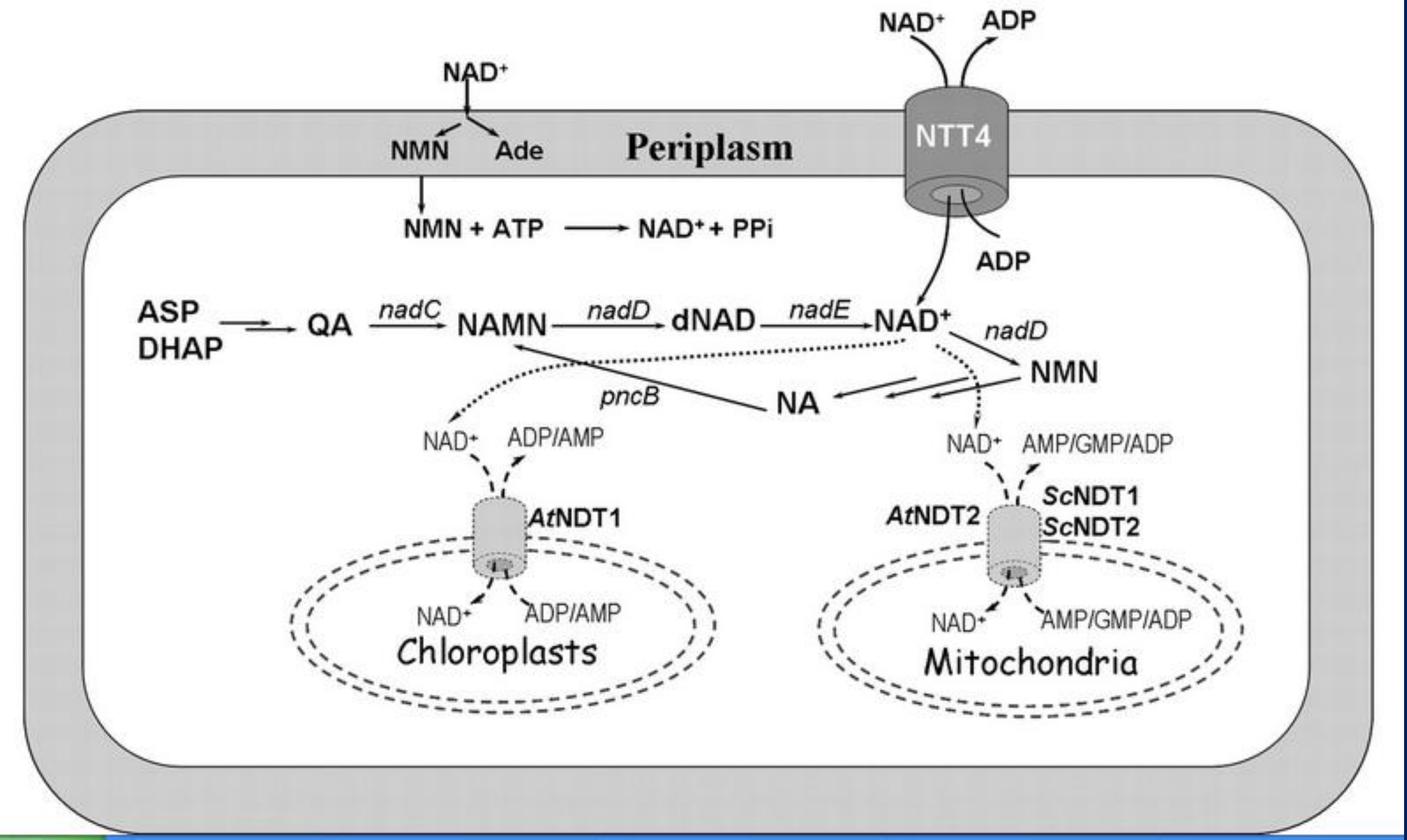
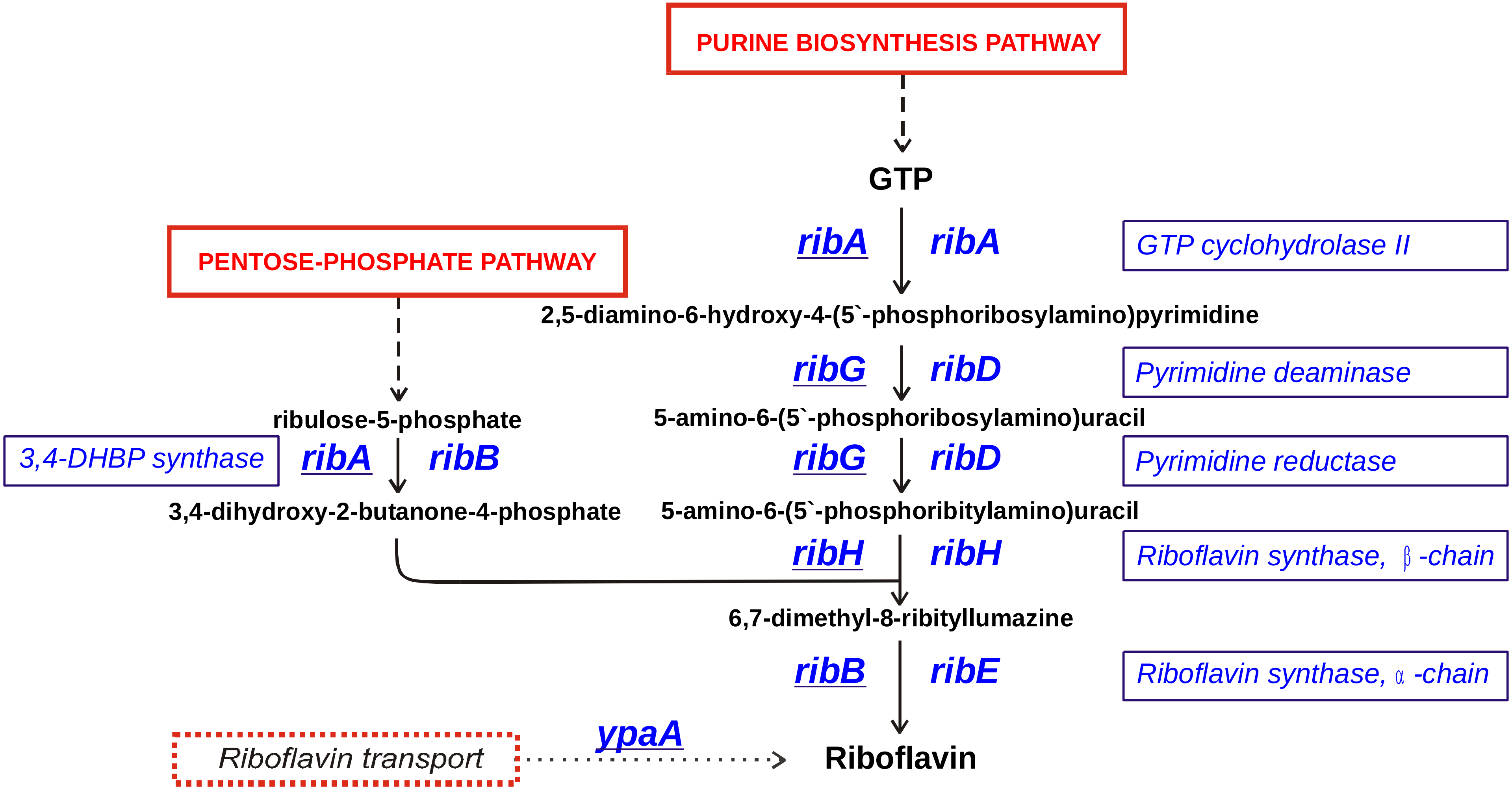
# Biosynthesis of NAD+ and NADP+ :
# A – prokaryotic; B - eukaryotic



# Transport of NAD



# Riboflavin biosynthesis pathway



PURINE BIOSYNTHESIS PATHWAY

**GTP**

**_ribA_** **_ribA_**

*GTP cyclohydrolase II*

PENTOSE-PHOSPHATE PATHWAY

**2,5-diamino-6-hydroxy-4-(5`-phosphoribosylamino)pyrimidine**

**_ribG_** **_ribD_**

*Pyrimidine deaminase*

**ribulose-5-phosphate**

**5-amino-6-(5`-phosphoribosylamino)uracil**

*3,4-DHBP synthase*

**_ribA_** **_ribB_**

**_ribG_** **_ribD_**

*Pyrimidine reductase*

**3,4-dihydroxy-2-butanone-4-phosphate**

**5-amino-6-(5`-phosphoribitylamino)uracil**

**_ribH_** **_ribH_**

*Riboflavin synthase, β -chain*

**6,7-dimethyl-8-ribityllumazine**

**_ribB_** **_ribE_**

*Riboflavin synthase, α -chain*

*Riboflavin transport*

**_ypaA_**

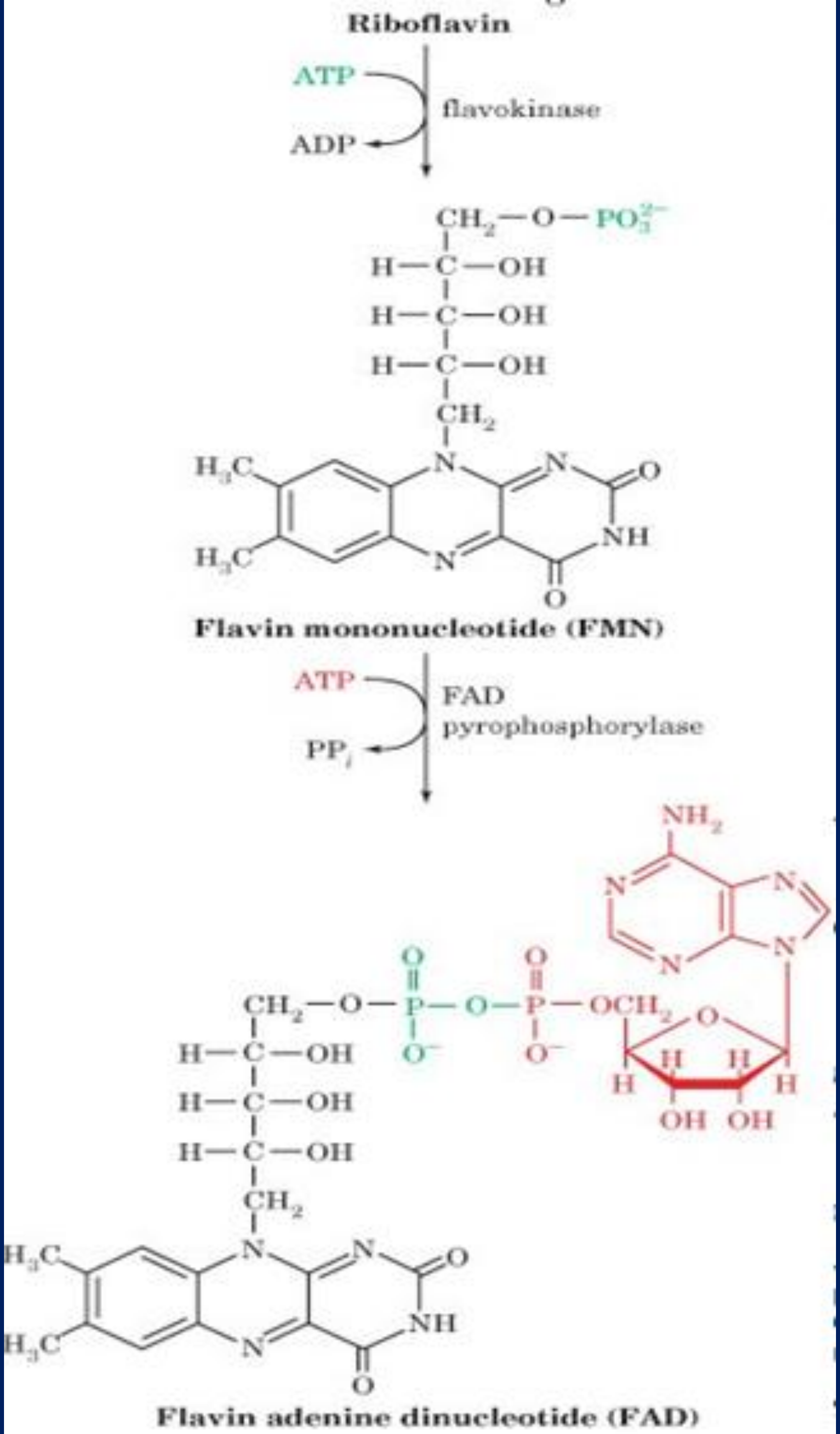
**Riboflavin**

# Biosynthesis of FMN and FAD from riboflavin

FAD is synthesized from riboflavin in a two-reaction pathway.

Flavokinase phosphorylates the 5’OH group to give FMN

FAD pyrophosphorylase catalyzes the next step (coupling of FMN to ADP).


Riboflavin
ATP
ADP
flavokinase
Flavin mononucleotide (FMN)
ATP
PP$_i$
FAD pyrophosphorylase
Flavin adenine dinucleotide (FAD)

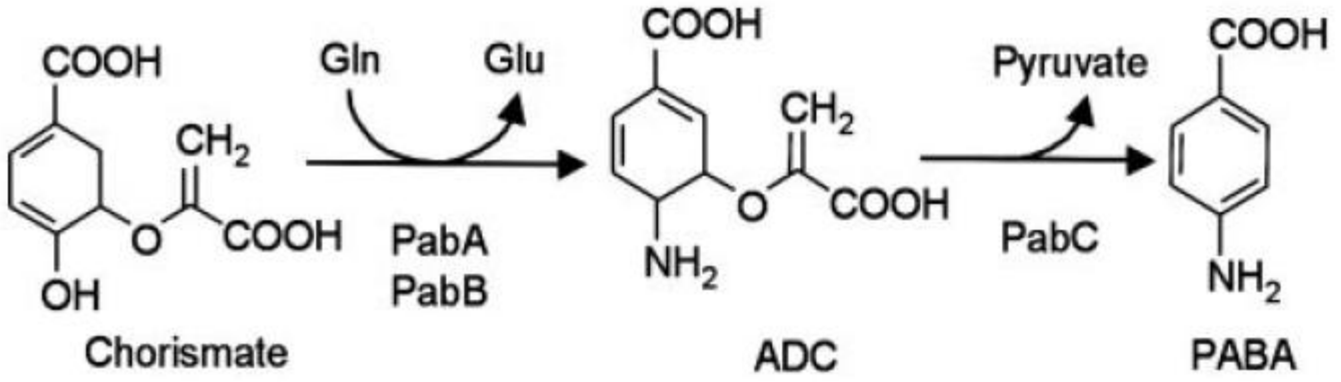
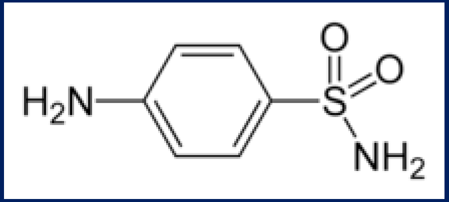
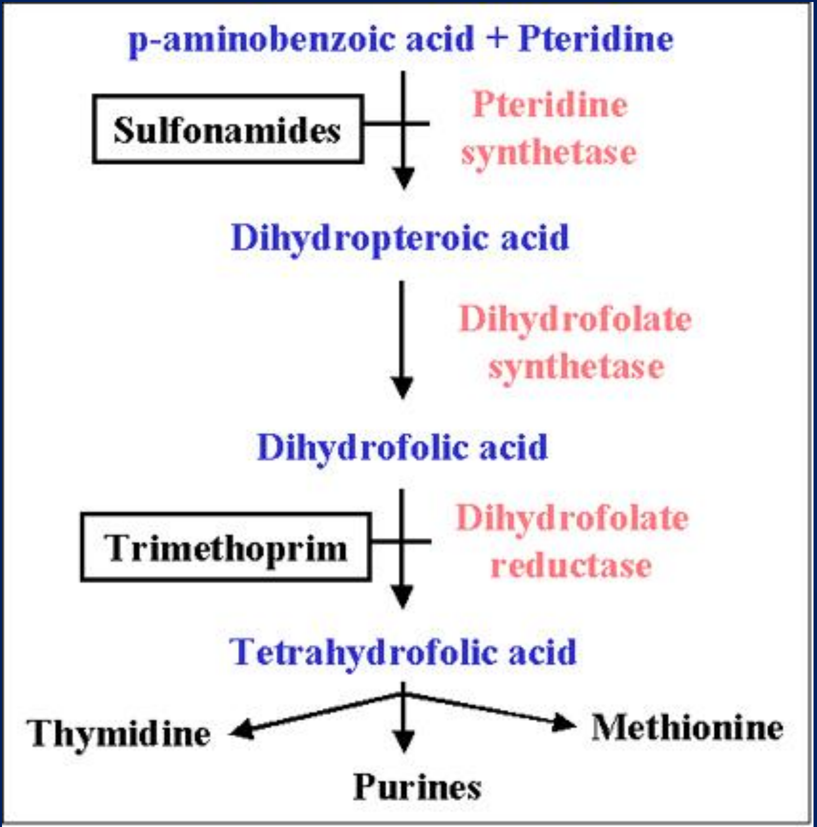


**Fig. 1.** The PABA biosynthesis pathway in *E. coli*. PabA and PabB associate to form the ADCS complex; PabC (ADC lyase) is a separate enzyme (4).

# Folate biosynthesis in bacteria

Synthesized by bacteria and thus the basis of sulfanylamide antibiotics





p-aminobenzoic acid + Pteridine

**Sulfonamides**

Pteridine synthetase

Dihydropteroic acid

Dihydrofolate synthetase

Dihydrofolic acid

**Trimethoprim**

Dihydrofolate reductase

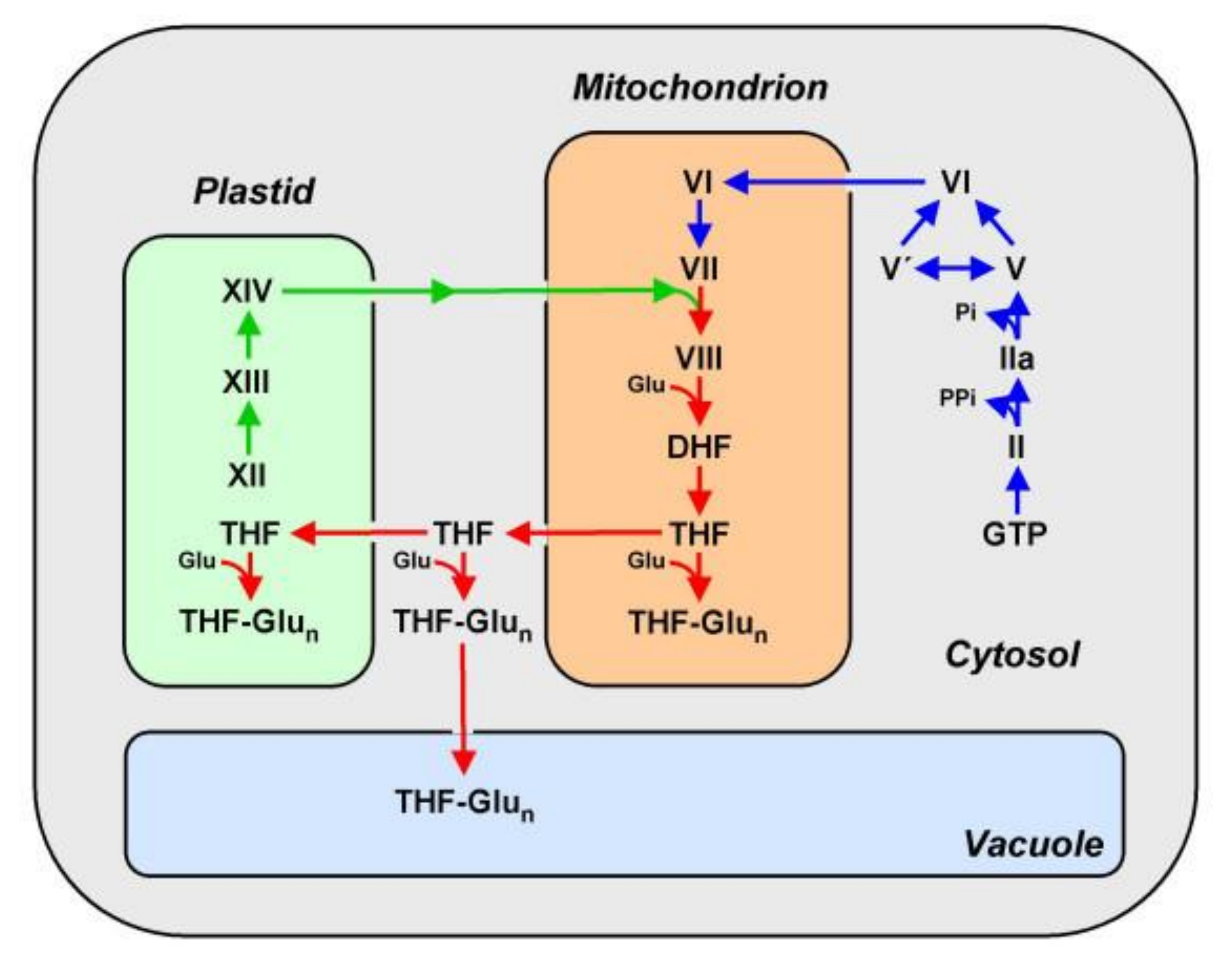
Tetrahydrofolic acid

Thymidine

Purines

Methionine

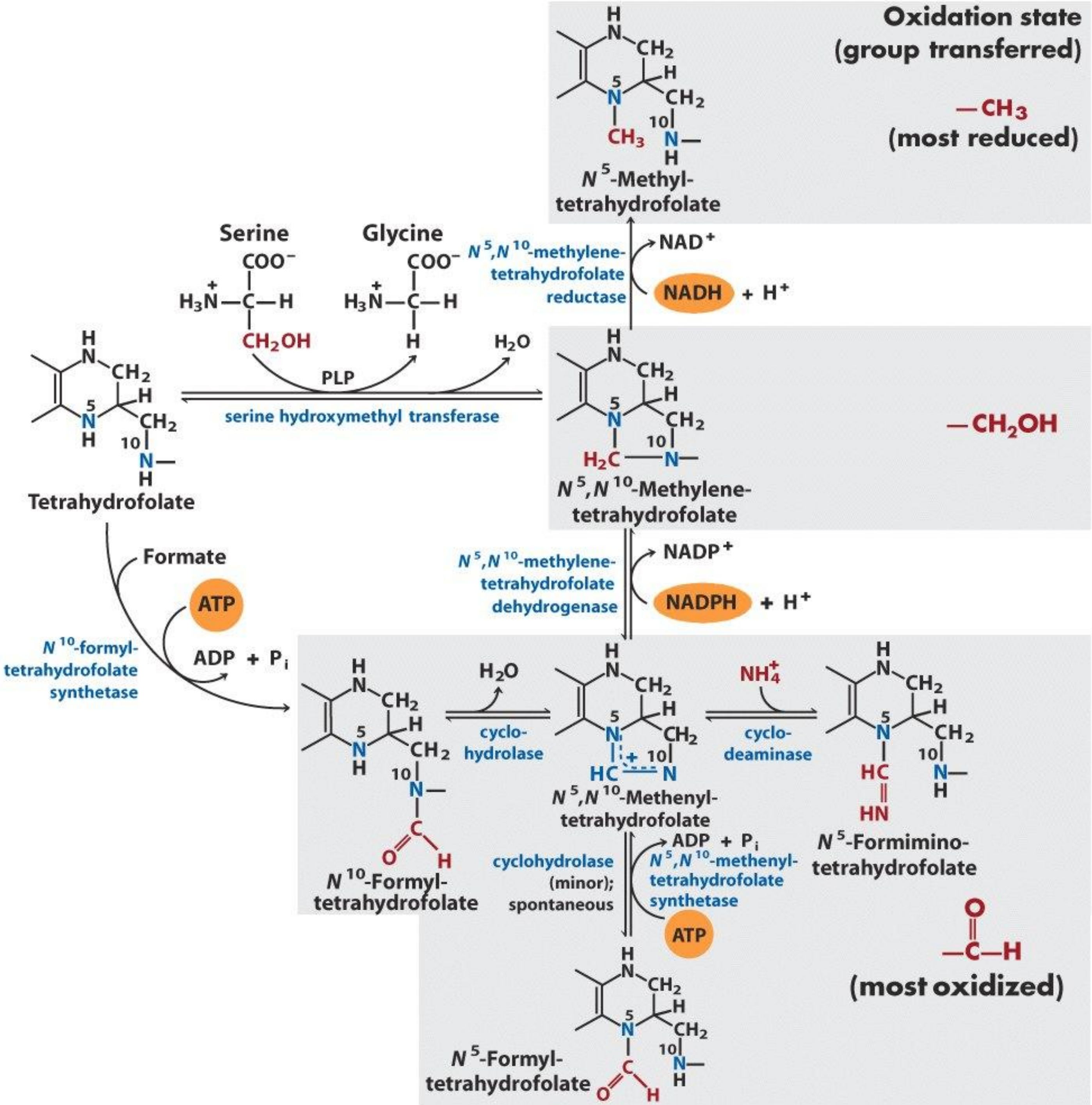
# Compartmentation of the folate synthesis pathway in plants



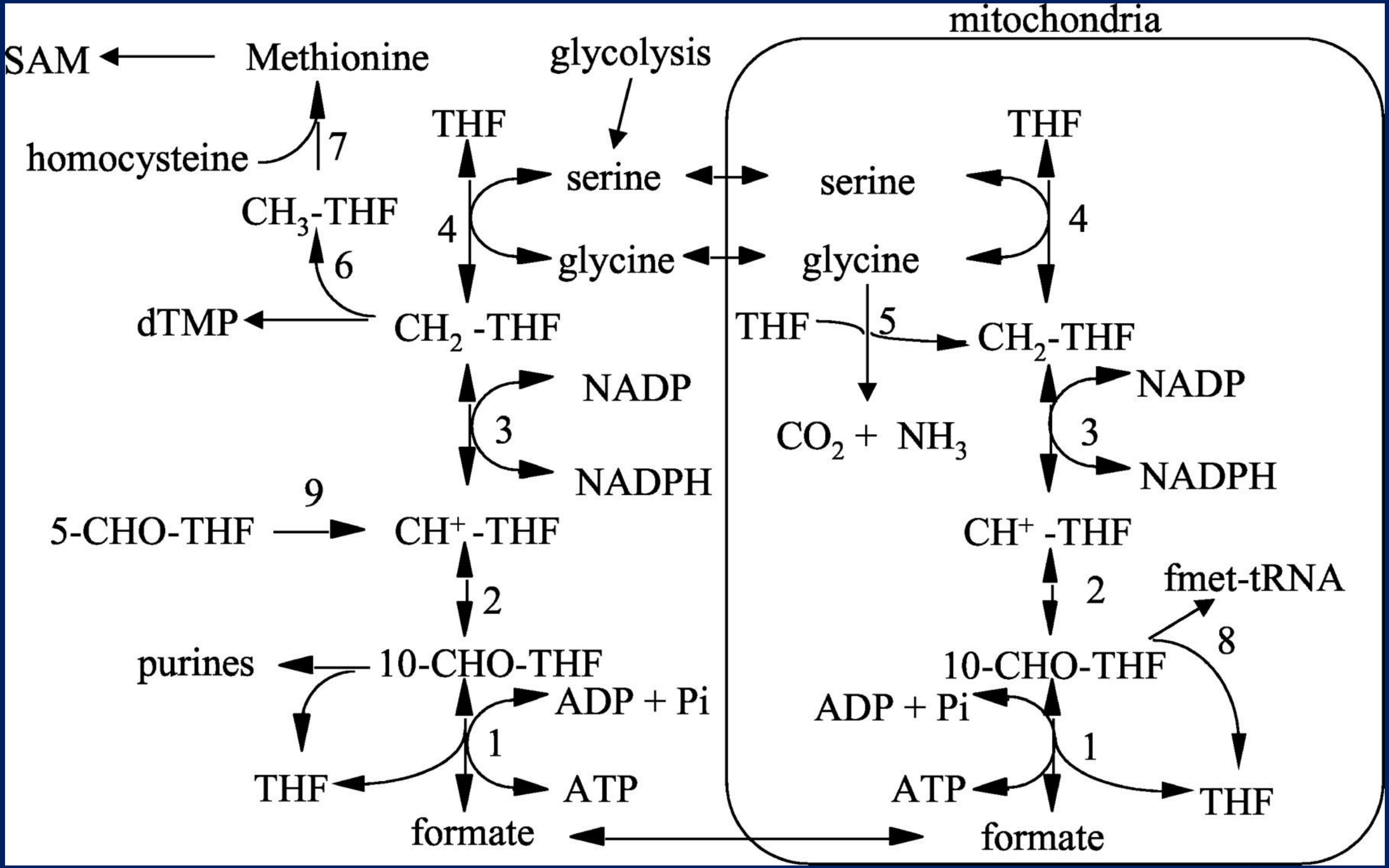
de Crécy-Lagard *et al. BMC Genomics* 2007 **8**:245

## Folate reactions

Transfer of 3 oxidation states of carbon


Oxidation state (group transferred)
—CH3 (most reduced)
N5-Methyl-tetrahydrofolate
N5,N10-methylene-tetrahydrofolate reductase
NAD+
NADH + H+
Serine
Glycine
PLP
H2O
serine hydroxymethyl transferase
Tetrahydrofolate
N5,N10-Methylene-tetrahydrofolate
—CH2OH
N5,N10-methylene-tetrahydrofolate dehydrogenase
NADP+
NADPH + H+
Formate
ATP
ADP + Pi
N10-formyl-tetrahydrofolate synthetase
N10-Formyl-tetrahydrofolate
cyclo-hydrolase
N5,N10-Methenyl-tetrahydrofolate
NH4+
cyclo-deaminase
N5-Formimino-tetrahydrofolate
cyclohydrolase (minor); spontaneous
N5,N10-methenyl-tetrahydrofolate synthetase
N5-Formyl-tetrahydrofolate
—C(=O)—H (most oxidized)

# Compartmentation of folate-mediated one-carbon metabolism in eukaryotes.



Prasannan P et al. J. Biol. Chem. 2003;278:43178-43187

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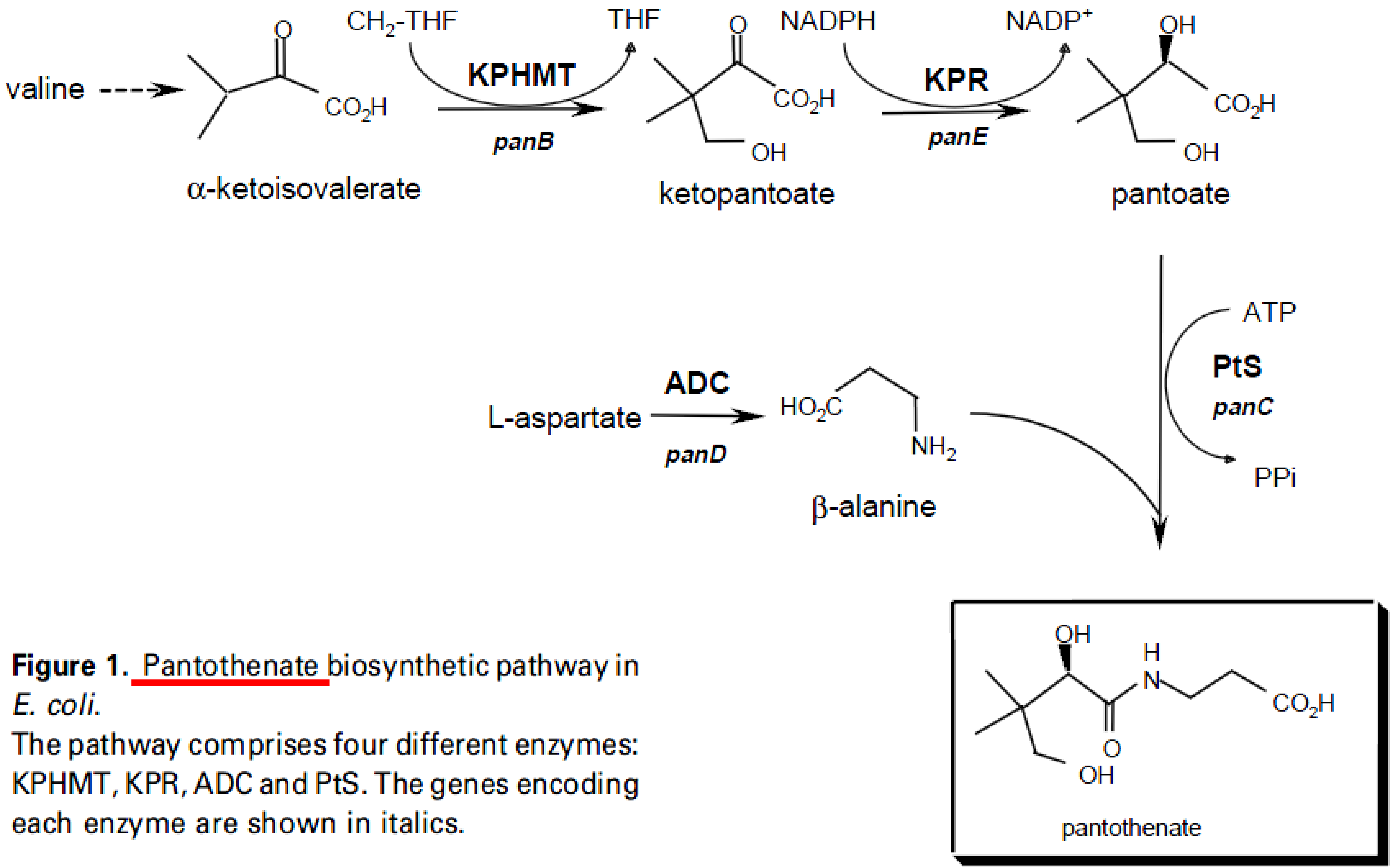
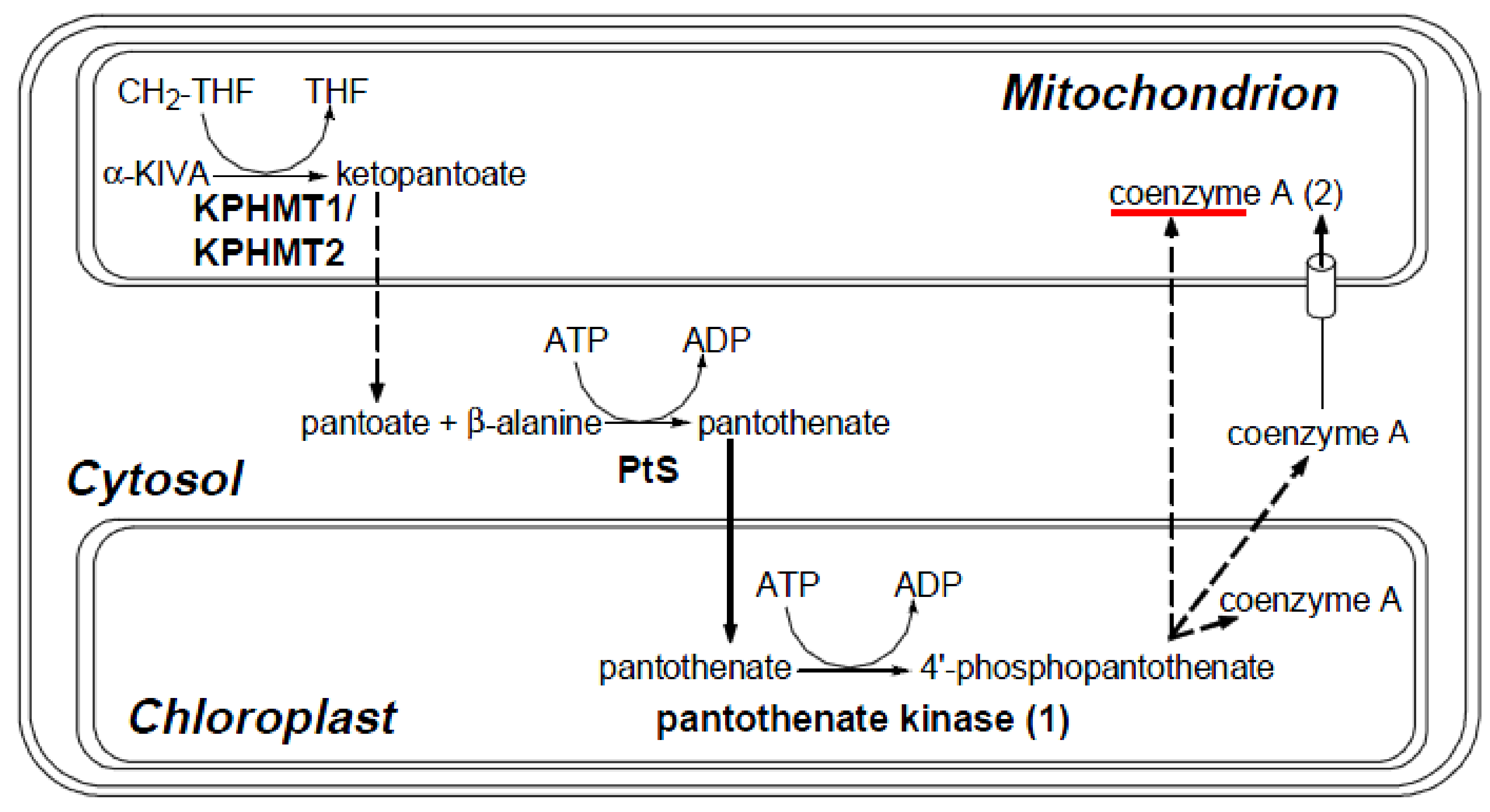


**Figure 1.** Pantothenate biosynthetic pathway in *E. coli.*

The pathway comprises four different enzymes: KPHMT, KPR, ADC and PtS. The genes encoding each enzyme are shown in italics.

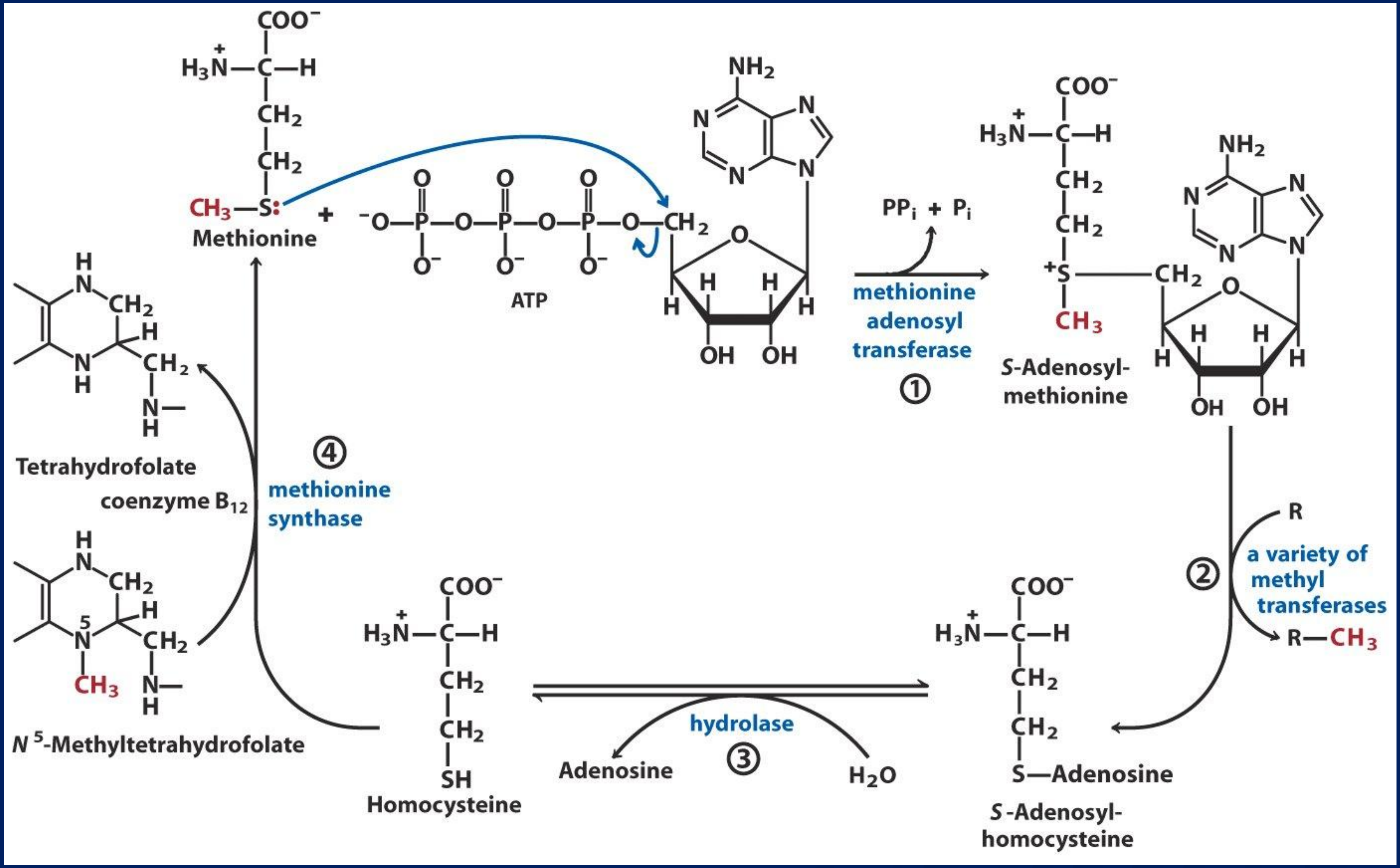


Schematic of the plant cell showing location of coenzyme A in different organelles and our current understanding of the subcellular distribution of pan enzymes. No plant KPR has yet been identified in plants and it is unclear whether ketopantoate or pantoate is exported from the mitochondrion. (1): Falk and Guerra (1993) reported that the first committed step to CoA biosynthesis, pantothenate kinase, was localised in chloroplasts of spinach; (2): CoA can be imported into plant mitochondria by a specific carrier in potato (Neuburger *et al.*, 1984).

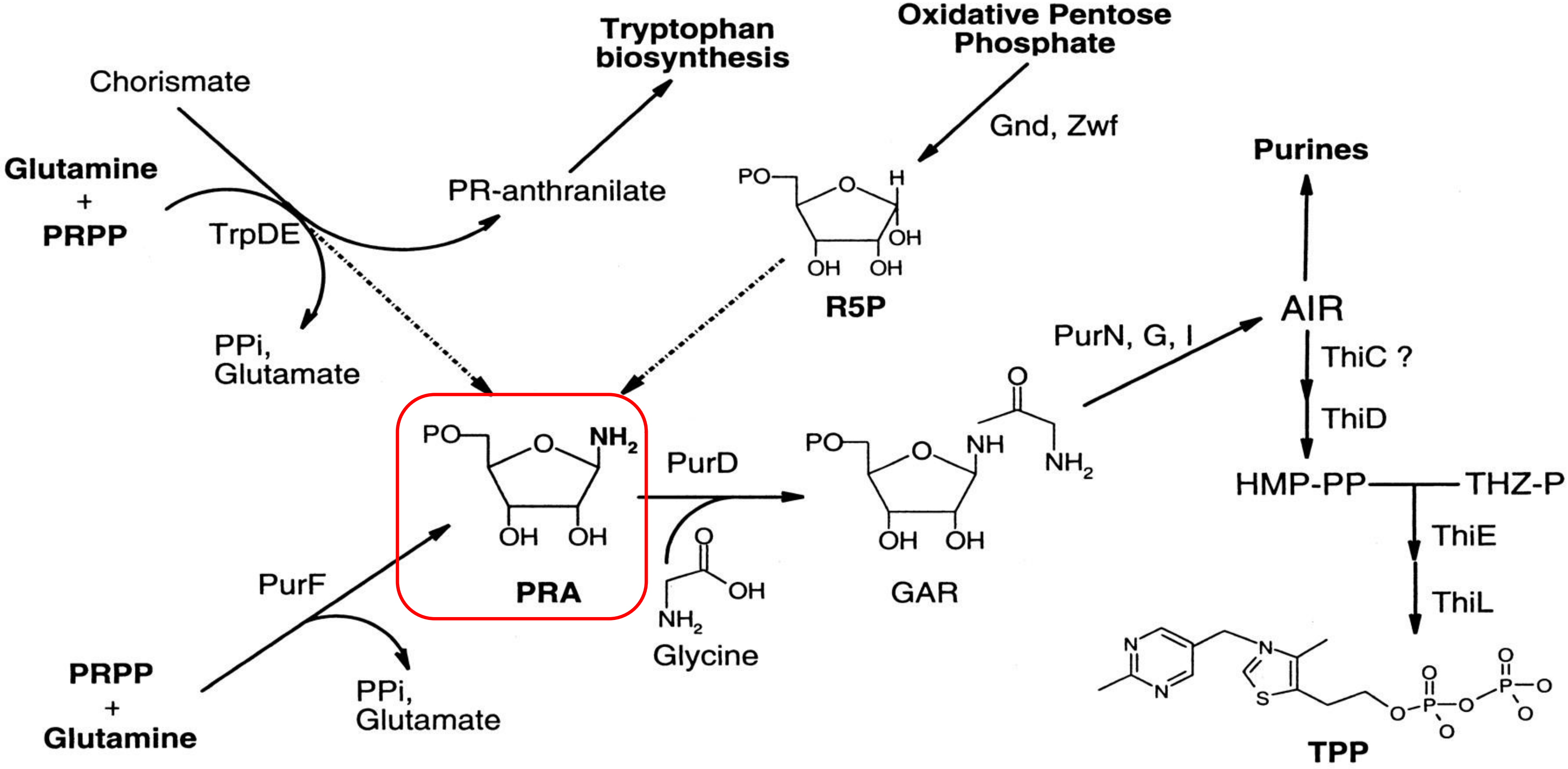
KIVA=ketoisovalerate

*The Plant Journal* (2004) **37**, 61–72

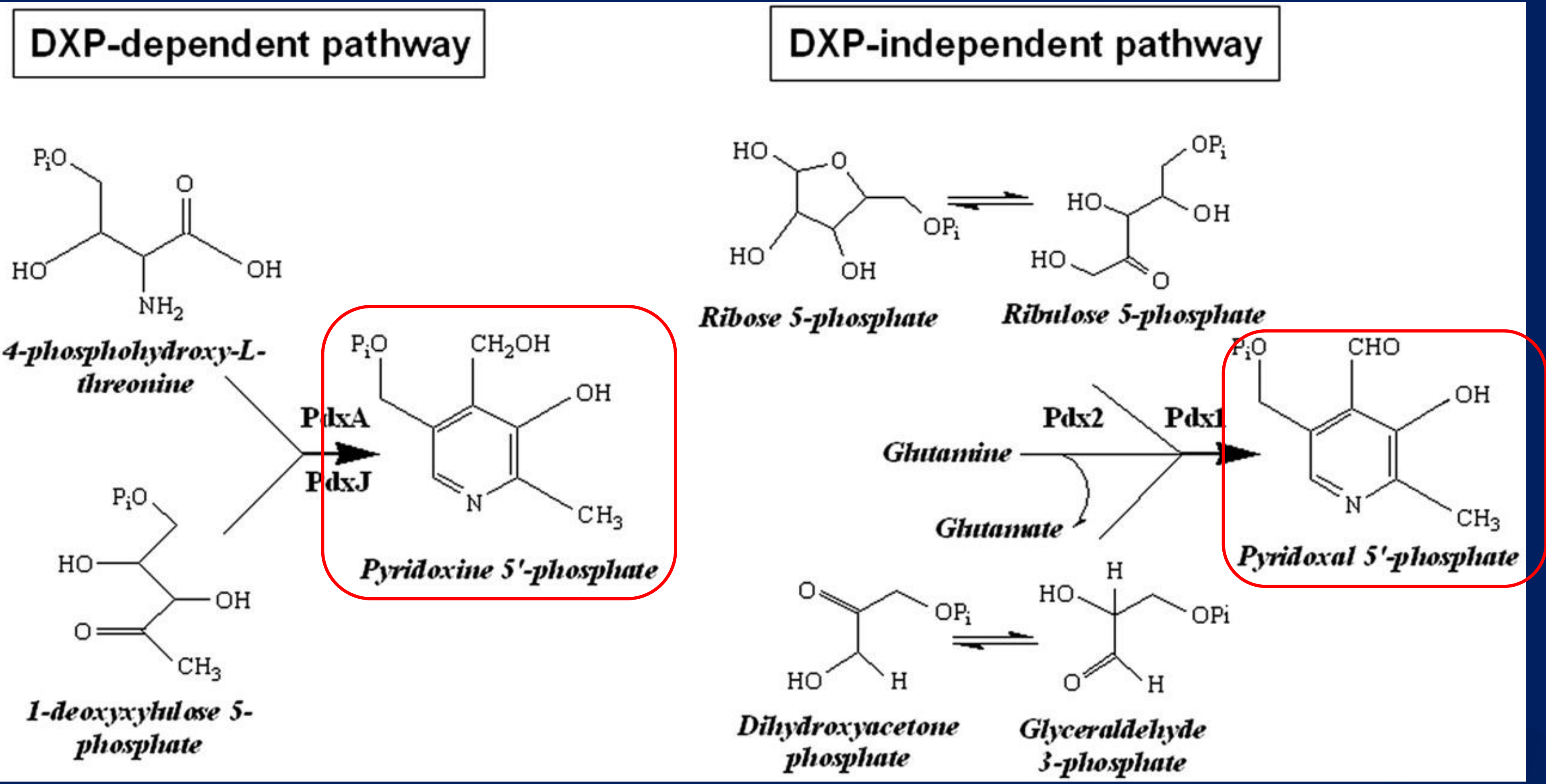
# **S-adenosyl methionine** (AdoMet, SAM), a powerful donor of methyl groups to RNA, DNA, proteins, lipids, neurotransmitters, carbohydrates



# Synthesis of Thiamine through phosphorybosylamine (PRA)



# Model depicting the DXP-dependent and -independent pathways of vitamin B6 biosynthesis.



Tambasco-Studart M et al. PNAS 2005;102:13687-13692

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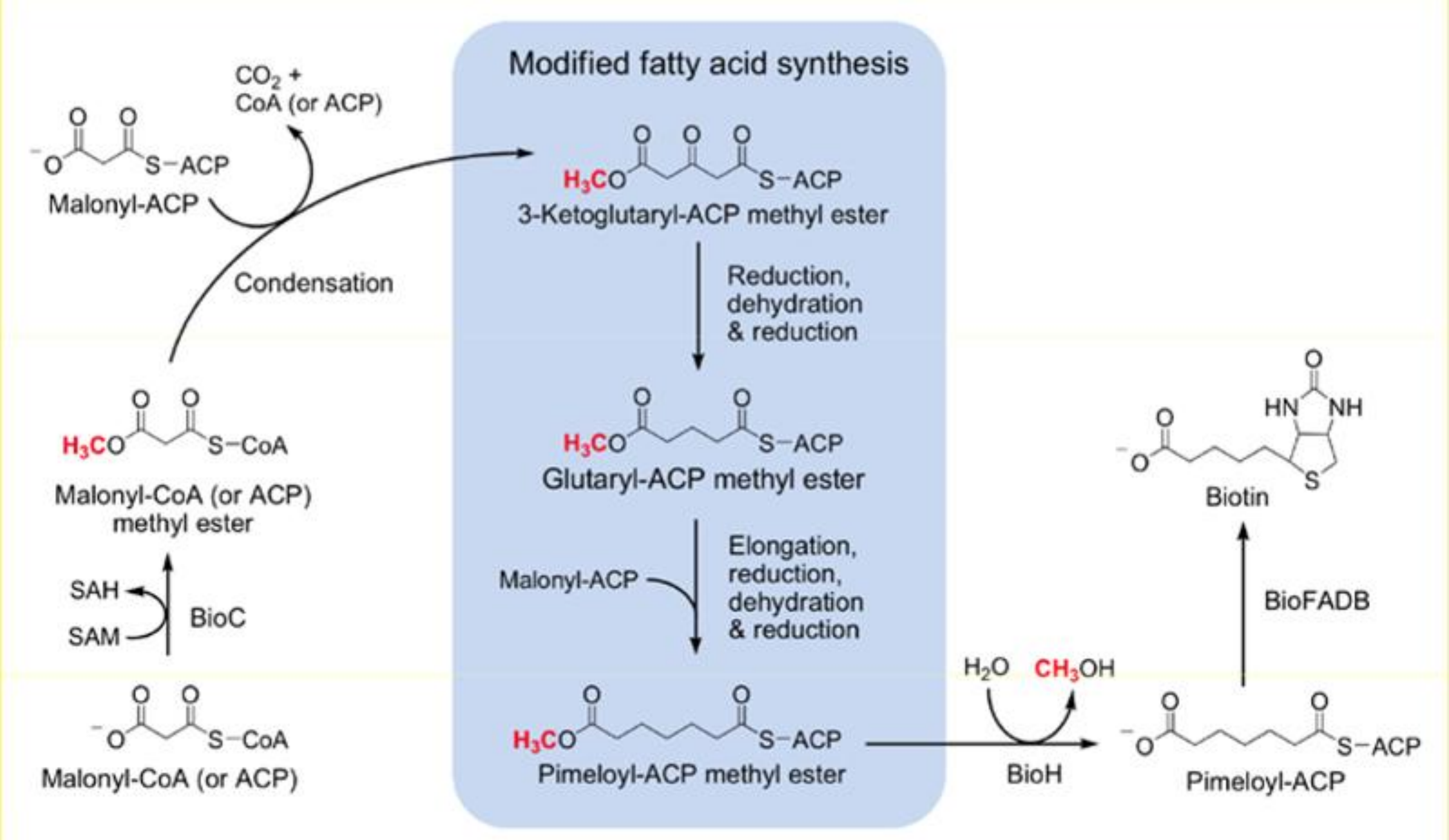
# Синтез биотина


Modified fatty acid synthesis
CO2 + CoA (or ACP)
S−ACP
Malonyl-ACP
H3CO
3-Ketoglutaryl-ACP methyl ester
Condensation
Reduction, dehydration & reduction
S−CoA
Malonyl-CoA (or ACP) methyl ester
Glutaryl-ACP methyl ester
Elongation, reduction, dehydration & reduction
Malonyl-ACP
HN
NH
Biotin
BioFADB
SAH
SAM
BioC
Malonyl-CoA (or ACP)
Pimeloyl-ACP methyl ester
H2O
CH3OH
BioH
Pimeloyl-ACP